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METHODS
of
PLANT BREEDING

BY

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METHODS OF PLANT BREEDING

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PREFACE

Plant breeding is an applied science that is carried out efficiently only through the application of other basic plant sciences. The rapid increase in knowledge of genetics since the rediscovery of Mendel's laws of heredity in 1900 and the application of these laws to plant breeding were essential steps in the development of plant breeding as a science. The contributions of cytogenetics in recent years have furnished, in many cases, a clear picture of genetic relationships based upon differences and similarities of chromosome morphology, structure, and function. Many economic plants are polyploids, and a knowledge of chromosome numbers, pairing behavior in crosses, and gene differences among related species and varieties is essential in building new varieties of plants with the characters desired by the grower and consumer. Physical and chemical methods of inducing changes in chromosome number and structure and of inducing gene changes are being developed. Satisfactory technics for inducing polyploidy in species and hybrids are available for certain types of plant breeding problems.

In order to evaluate a variety, it is necessary to compare it with varieties of known performance. The comparisons made by the plant breeder are extensive, and frequently only a few replications can be grown. The development of adequate statistical methods has aided greatly in making reliable comparative trials. Experimental methods of making reliable comparisons are one of the tools of the plant breeder.

Methods have been devised in many cases for differentiating quality, for a determination of the relative value of different characters, including chemical properties, that make it possible under conditions of controlled pollination to select for the characters desired. In problems of breeding for disease resistance, a knowledge of the genetics of the pathogen is as essential as that of the crop plant itself. With each individual plant, information regarding available varieties, their characters, and

their wild relatives furnishes a basis for the combination of genes desired by the breeder. For diseases caused by pathogens it is equally important to know the probable mode of origin of new strains of the organism, and the number, distribution, and genetic nature of the strains present in the region where the crop plant is to be grown.

The subject matter presented in "Methods of Plant Breeding" has been used in both undergraduate and graduate courses at the University of Minnesota. The undergraduate course is taught only to junior and senior students. The graduate courses are given for the purpose of teaching standardized methods of breeding for particular categories of breeding problems and to present the current viewpoint when the most desirable method of breeding is not so well known. This is with the belief that each of the various methods of hybridization, including the pedigree method of selecting during the segregating generations, the bulk method with self-pollinated plants, the backcross method and convergent improvement, has certain advantages and disadvantages that make it desirable under some conditions and less desirable for other breeding problems.

A great deal of information is available regarding the genetics of many crop plants, and added information is being obtained very rapidly. It seems unwise to attempt a complete review of the present status of the genetics of many crop plants, since the information available is very extensive and such a review would be out of date almost as soon as it was published. Concise reviews of the mode of inheritance of important characters of the small grains, flax, and corn have been included to illustrate the value to the breeder of a knowledge of inheritance as an aid in planning the breeding program. These should be supplemented by similar reviews of inheritance for those crop plants that are of greatest value for each class of students who use the book.

The present status of corn breeding, a rather typical cross-pollinated plant, has been reviewed in considerable detail, since many of the studies made with corn and the results obtained are basic to an understanding of principles of breeding other cross-pollinated plants.

Methods of field-plot technic, experimental design, and statistical analysis with particular reference to plant-breeding problems have been discussed, including some of the newer methods. The

necessary statistical tables have been included with the permission of the original publishers.

The authors are indebted to Professor R. A. Fisher and to Messrs. Oliver and Boyd, of Edinburgh, for permission to reprint completely or in abridged form Appendix Tables I, III, and IV from their book "Statistical Methods for Research Workers," 7th Ed. (1938) and to Professor George W. Snedecor and his publishers, Iowa State College Press, for permission to reprint Appendix Table II from their book "Statistical Methods," 3d Ed. (1940). Professor Snedecor has given permission to reprint Appendix Table V, and Dr. C. I. Bliss has given permission to reprint Appendix Table VI.

Various coworkers have read particular chapters and have made helpful suggestions. Particular thanks are due to Dr. C. R. Burnham for suggestions regarding the chapters on Genetics and on Inheritance in Maize; to Dr. E. R. Ausemus for suggestions regarding the chapter on Inheritance in Wheat; to Dr. F. A. Krantz for helpful suggestions regarding potato improvement; to A. G. Tolaas for information regarding potato-seed certification; and to Dr. C. H. Goulden for reviewing the chapters on Field-plot Technic and on Statistical Methods. Dr. H. M. Tysdal kindly furnished unpublished information regarding the effects of self-pollination in alfalfa. In problems relating to disease resistance, suggestions by Dr. J. J. Christensen and M. B. Moore have been specially helpful. "Breeding Crop Plants," by Hayes and Garber, has been used freely. The writers, however, accept full responsibility for the viewpoints presented.

H. K. HAYES,
F. R. IMMER.

UNIVERSITY OF MINNESOTA,
February, 1942.

CONTENTS

PAGE

PREFACE v

CHAPTER I

THE ROLE OF PLANT BREEDING 1

The value of plant breeding—Genetic principles are the basis of scientific breeding—Breeding spring wheat resistant to stem rust—Corn breeding—Potato improvement.

CHAPTER II

THE GENETIC AND CYTOGENETIC BASIS OF PLANT BREEDING 11

Chromosome number (haploid) in common crop plants—Polyploids in relation to plant breeding—Some applications of genetics to plant breeding—Colchicine as a polyploidizing agent.

CHAPTER III

MODE OF REPRODUCTION IN RELATION TO BREEDING METHODS 39

The asexual group—The sexual group—Self-pollination leads to homozygosis—The effects of self-pollination in the often cross-pollinated group. Effects of self-fertilization in cross-pollinated plants—Heterosis and its explanation—A classification of methods of breeding sexually propagated plants.

CHAPTER IV

TECHNICS IN SELFING AND CROSSING 60

Corn—Wheat, oats, and barley—Rye—Flax—Cotton—Sorghum—Rice—Potato—Pumpkin and squash—Onion—Red Clover—Alfalfa and sweet clover—Grasses.

CHAPTER V

THE PURE-LINE METHOD OF BREEDING NATURALLY SELF-POLLINATED PLANTS 74

Early studies—The pure-line theory—The pure-line theory in its application—Methods of improving self-fertilized plants by individual-plant selection—Utilization of introductions—Pedigree selection within adapted varieties—Cooperative tests and distribution of promising lines—Illustrations of valuable varieties of self-pollinated plants produced by application of the pure-line theory.

CHAPTER VI

HYBRIDIZATION AS A METHOD OF IMPROVING SELF-POLLINATED PLANTS 86

Some studies before 1900—Development of methods since 1900—Breeding improved varieties of barley—Breeding by hybridization

PAGE

—Object of crossing—Selection of parental material—Technic of crossing—Handling the hybrid material—Methods of breeding—Pedigree method—Bulk method—Backcross method—Multiple crosses—Combining ability.

CHAPTER VII

THE BACKCROSS METHOD OF PLANT BREEDING 101

Genetic expectations from backcrossing—Cantaloupes resistant to powdery mildew, *Erysiphe cichoracearum*—Breeding bunt-resistant wheats—Breeding rust-resistant snapdragons—Studies at the Minnesota station—Disease resistance in wheat—Illustrations with corn.

CHAPTER VIII

BREEDING FOR DISEASE AND INSECT RESISTANCE 113

The importance of disease resistance—Methods of breeding for disease and insect resistance—The search for resistant material—Artificial production of ephiphytotics—Black stem rust of wheat, oats, and barley (*Puccinia graminis*)—Leaf rust of wheat (*P. triticina*) and crown rust of oats (*P. coronata*)—Bunt of wheat (*Tilletia tritici*)—Loose smut of oats (*Ustilago avenae*); covered smut of oats (*U. levis*)—Covered smut of barley (*U. hordei*) and intermediate smut of barley (*U. medians*)—Flax rust (*Melampsora lini*)—Flax wilt (*Fusarium lini*)—Fusarial head blight (scab) of wheat and barley—Corn smut (*U. zeae*)—Loose smut of sorghum (*Sphacelotheca cruenta*) and covered smut of sorghum (*S. sorghi*)—Head smut of sorghum and corn (*S. reilianum*)—Hessian-fly injury of wheat—Methods of breeding—Study of fundamental problems.

CHAPTER IX

INHERITANCE IN WHEAT 129

Glume shape—Awnedness—Chaff characters—Seed characters—Spike density—Spring and winter habit—Stem-rust reaction—Bunt resistance—Other problems of disease resistance—Quantitative characters.

CHAPTER X

INHERITANCE IN OATS 141

Species groups—Inheritance of characters in crosses between 42 chromosome species—Differences in awn development—Color of grain—Hulled vs. hull-less—Spreading vs. side panicle—Pubescence—Disease reactions—Stem rust—Crown rust—Correlated inheritance of reaction to three diseases—Smuts—Quantitative characters.

CHAPTER XI

INHERITANCE IN BARLEY 152

Classification and genetics of barley species—Chromosome number in genus *Hordeum*—Linkage groups—Internode length in the

PAGE

rachis of the spike—Reaction to *Helminthosporium sativum*—Reaction to stem rust—Resistance to mildew—Interaction of factors affecting quantitative characters.

CHAPTER XII

INHERITANCE IN FLAX 165

Factors for flower and seed color in common flax—Dehiscence of the bolls—Smooth vs. ciliate septa—Weight of seed and oil content—Inheritance of quality of oil—Disease resistance—Wilt resistance—Resistance to rust.

CHAPTER XIII

METHODS OF SELECTION FOR SPECIAL CHARACTERS 175

Quality tests in wheat—Wheat-meal fermentation time test—Cold-resistance tests with wheat—Shattering in wheat—Dormancy in relation to breeding—Lodging in small grains and corn—Drought studies with corn—Inducing biennial sweet clover to flower the first year—Determination of coumarin content in sweet clover—Method of determining hydrocyanic acid content of single plants of sudan grass.

CHAPTER XIV

DEVELOPMENT OF METHODS OF CORN BREEDING 187

Selection without controlled pollination—Early studies of self- and cross-fertilization with corn—Controlled pollination methods—Breeding improved inbred lines—The pedigree method of selection in the segregating generations after crossing inbreds—Genetic diversity—The backcross—Convergent improvement.

CHAPTER XV

INHERITANCE IN MAIZE 215

Origin and classification—The pod corns—The flint corns—The popcorns—The dent corns—The flour corns—The sweet corns—The waxy corns—Endosperm characters—Chlorophyll variations—Plant color—Glossy seedlings—Linkage studies with maize—Inheritance of quantitative characters—Linkage of factors for row number with genes at known loci—Inheritance of smut reaction—Inheritance of combining ability—Inheritance of other important characters.

CHAPTER XVI

CONTROLLED POLLINATION METHODS OF BREEDING CROSS-POLLINATED PLANTS 242

Effects of self-fertilization—Inheritance of self-incompatibility—Methods of breeding—Outline for improvement of cross-pollinated plants by controlled pollination methods.

PAGE

CHAPTER XVII

SEED PRODUCTION 263

Selecting the variety—First increase of seed of a new variety—Seed certification and registration—The Canadian Seed Growers' Association—The International Crop Improvement Association—Description of seed classes—The Minnesota plan for certain crops—Seed (tubers) certification for potatoes.

CHAPTER XVIII

SOME COMMONLY USED MEASURES OF TYPE AND VARIABILITY 280

Definition of statistical constants—Calculation of mean—Standard error. variance, and coefficient of variability—Correlation coefficient—Comparison of differences by the *t* test.

CHAPTER XIX

FIELD-PLOT TECHNIC 289

Crop rotation for experimental fields—Soil heterogeneity—Competition—Size and shape of plots—Replication—Methods of making yield trials used in Minnesota.

CHAPTER XX

RANDOMIZED BLOCKS, LATIN SQUARES, AND χ^2 TESTS 307

Tests in randomized blocks—Latin squares—Estimating the yield of a missing plot—Split-plot experiments—Chi square (χ^2) tests.

CHAPTER XXI

CORRELATION AND REGRESSION IN RELATION TO PLANT BREEDING. . . 325

Simple correlation—Linear regression—Means and differences of correlation coefficients—Partial correlation—Multiple correlation.

CHAPTER XXII

MULTIPLE EXPERIMENTS, METHODS OF TESTING A LARGE NUMBER OF VARIETIES, AND THE ANALYSIS OF DATA EXPRESSED AS PERCENTAGES 339

Multiple experiments in randomized blocks—Comparison of varieties in different experiments where the same check varieties are grown—Simple lattice experiments—Triple lattice experiments—Analysis of data expressed as percentages.

LITERATURE CITATIONS 381

GLOSSARY 401

APPENDIX 411

INDEX. 425

METHODS OF PLANT BREEDING

CHAPTER I

THE ROLE OF PLANT BREEDING

There is a growing appreciation of the value of plant breeding as a means of obtaining new or improved plant forms adapted to a wide variety of uses. Although most important food plants had been brought under cultivation before the dawn of recorded history, there remains at present almost an unlimited opportunity to improve the varieties of plants available for various agricultural uses and in some cases greatly to modify their characters. The primary purpose is to obtain or produce varieties or hybrids that are efficient in their use of plant nutrients, that give the greatest return of high-quality products per acre or unit area in relation to cost and ease of production, and that are adapted to the needs of the grower and consumer. It is of great importance also to obtain varieties that are able to withstand extreme conditions of cold or drought or that have resistance to pathogenic diseases or insect pests. Such qualities help materially to stabilize yields by controlling extreme fluctuations.

Although the art of plant breeding, *i.e.*, the ability to discern fundamental differences of importance in the plant material available and to select and increase the more desirable types, is a great asset to the breeder, there is general appreciation that plant breeding, efficiently carried out, is to a large extent dependent on fundamental training in the biological sciences.

Some of the more important phases of this training may be summarized as follows:

1. A knowledge of genetic and cytogenetic principles.
2. A knowledge of the characteristics of the crop to be improved, including its wild relatives.
3. Information regarding the needs of the grower.
4. A knowledge of special technics adapted to the solution of particular problems.

5. A knowledge of the principles of field-plot technic.

6. A knowledge of the principles involved in the design of experiments and the statistical reduction of data.

The purpose of this book is to summarize the methods of breeding that have been developed for particular categories of crop plants, to explain the reason why particular methods are chosen for certain types of crop-improvement problems, and to give methods of field-plot technic and of statistical analysis that are adapted for particular uses. Although individual crop problems will be used for illustration, there will be no attempt to summarize the work that has been done or that needs to be done with individual crop plants except as these facts may aid in understanding types of problems. Emphasis will be placed on methods of breeding and principles underlying their use.

THE VALUE OF PLANT BREEDING

E. F. Gaines (1934), of the Washington Agricultural Experiment Station, has made the statement that the practical results of genetic research on disease resistance in plants has helped to popularize genetics with the general public. The illustration of breeding stem-rust-resistant wheat given later in the chapter emphasizes the value of cooperation in research and the need of intensive study in order to solve underlying principles and thus make possible the solution of complex problems and the development of the desired varieties.

In discussing the importance of a knowledge of genes and their control, the following statements have been made by Muller:

> Organisms are found to be far more plastic in their hereditary basis than has been believed, and we may confidently look forward to a future in which—if synthetic chemistry shall not have displaced agriculture—the surface of the earth will be overlaid with luxuriant crops, at once easy to raise and to gather, resistant to natural enemies and climate, and readily useful in all their parts.
>
> This work is a far vaster one than the layman ordinarily realizes, for there are many thousands of wild species of plants whose varied potentialities must be tested, and many species both wild and cultivated already contain hundreds of varieties and thousands of individual differences. By means of laborious crossing methods, these diverse types may be combined and recombined within wider limits, and so a virtually endless succession of specialized hybrid forms may be produced, differentiated

into local geographical races each having characteristics especially suited to its peculiar conditions of cultivation and to the needs of the district. When to the potentialities of hybridization are added those that will appear as new hereditary types arising by mutation, the path of change and adaptation is seen to be indeed limitless.

GENETIC PRINCIPLES ARE THE BASIS OF SCIENTIFIC BREEDING

Many years ago Raymond Pearl emphasized the fact that with self-pollinated crop plants the plant breeder was using Mendel's laws as a direct working guide. Illustrations by the score could be given to show how particular types of genetic knowledge have been and are being used as a basis for a planned crop-improvement program. A few illustrations will be given to show the extent to which a knowledge of the genetics of a particular crop is essential in the development of a logical breeding program.

Breeding Spring Wheat Resistant to Stem Rust.—One of the principal cooperative projects at Minnesota since 1915, in which agronomists, plant geneticists, cereal chemists, and plant pathologists have all played their parts, has been the development of rust-resistant varieties of spring wheat of desirable agronomic type and of satisfactory milling and baking quality. This research program has been carried on through cooperation between workers in the Minnesota Experiment Station and the U. S. Department of Agriculture.

In these studies, artificial epidemics of stem rust have been developed both under field conditions and in the greenhouse. The rust nursery in the field has consisted of several thousand rows yearly. During the early period of this study, resistant vulgare wheats were unknown. The present nursery has such a preponderance of strains of vulgare wheats highly resistant to stem rust that it is necessary to plant a considerable amount of susceptible host material throughout the nursery in order that rust may develop sufficiently so that a satisfactory spread of the disease may be made possible. The development of rust-resistant strains has been accomplished by obtaining resistance from the Emmer group and by combining this resistance with the desirable agronomic characters of vulgare wheats through a series of crosses and selections.

At the present time much remains to be known about various phases of stem-rust resistance in wheat, but many problems have

been solved. Some of the steps leading to our present position may be mentioned.

1. The mode of inheritance of particular types of reaction to stem rust has been determined in both the greenhouse and field. The most important practical result of these studies is the conclusion that resistance to all races of stem rust of wheat in the stage from heading to maturity may be dependent upon only a single or a few genetic factors.

2. The pathogene causing the disease *Puccinia graminis tritici* Eriks. & Henn. is composed of numerous forms, called physiologic races, that can be differentiated by their manner of reaction with a series of wheat varieties and species known as differential hosts, this separation being made primarily on the basis of seedling reaction. A wheat variety resistant to a particular race of rust in the seedling stage is resistant to the same race in all stages of plant growth under field conditions. Physiological resistance in the seedling stage is of such a nature that a wheat may be immune from one race of rust and susceptible to another. As an illustration, Kanred winter wheat and some hybrid derivatives having Kanred as an ancestor are immune from certain races and highly susceptible to others. This knowledge explains the reason why Kanred winter wheat and derivatives may be highly resistant in one season and highly susceptible in another.

3. A knowledge of the causes of resistance has been of major importance. Thus, the resistance of Kanred is physiological and acts only against particular races of rust. A second type of resistance under field conditions to many races of rust as the plants approach maturity, called mature-plant resistance, appears to be simply inherited. The exact cause of this type of resistance is unknown. Some have suggested that morphological and functional causes may be responsible. Others have given evidence indicating that this does not seem to be the explanation. Mature-plant resistance is inherited, in some cases, in a simple Mendelian manner, especially where the varieties Hope and H44 are used as the resistant parents.

4. It has been learned also that extreme conditions of environment may cause an apparent breaking down of resistance to a particular disease. For example, a plant genotypically resistant to stem rust, if infected with loose smut, may be completely susceptible to rust. This conclusion seems essential in a logical

viewpoint of disease resistance in plants. No one expects that a potentially high-yielding variety will give high yields under unfavorable conditions. Extreme conditions of environment may strongly modify reaction to disease by modifying the character that, under normal conditions, is responsible for the resistance to that particular disease.

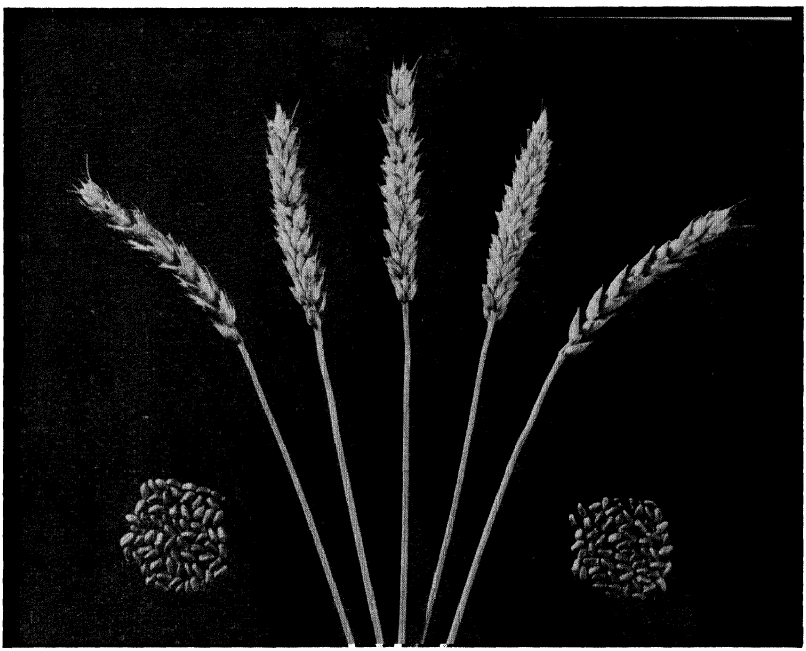


FIG. 1.—Thatcher wheat was first released in Minnesota in the spring of 1934. There were approximately 12,000,000 acres grown in Canada in 1940 and 5,500,000 acres in the United States. The estimate has been made that Thatcher has given an annual increase in farm income to the Minnesota farmer of 2 million dollars.

Thatcher wheat (Hayes *et al.* 1936), first introduced in the spring of 1934 in Minnesota, is now the most widely grown stem-rust-resistant wheat, being the major spring wheat grown in 1939 in the United States in the eastern and central sections of the spring-wheat area. Thatcher is grown extensively also in Canadian provinces where stem rust is most severe. It withstood the stem-rust epidemics of 1935, 1937, and 1938 when susceptible varieties of spring wheats were very severely injured. Thatcher excels in yielding ability, strength of straw, and milling and baking quality but is somewhat less satisfactory in weight per

bushel than some other varieties, partly because of its small size of seed and its susceptibility to scab and leaf rust. The latter disease was epidemic in 1938 in the spring-wheat area.

Thatcher is the product of a double cross between (Iumillo *durum* × Marquis) × (Marquis × Kanred). From the first cross of Iumillo × Marquis and selection during F_2 to F_5, 4 related strains were obtained with 21 pairs of chromosomes, vulgare type of plant, and resistance to stem rust. It is of interest that no plants were obtained in F_2 that had this combination of characters but that from over one thousand F_3 lines there was one line that contained several plants that were of vulgare type that were resistant to stem rust. From the cross of Marquis × Kanred, a considerable number of spring wheats were selected that were homozygous for the immunity of Kanred to several rust races. Thatcher was selected from a cross between the more promising strains of these two single crosses. The Thatcher variety combines field resistance to many physiologic races of stem rust with seedling resistance to the races to which Kanred is immune. It was the first successful attempt to transfer rust resistance from the Emmer group with a haploid chromosome number of 14 to the vulgare group ($n = 21$).

McFadden (1930) was the first to develop vulgare wheats with near immunity to stem rust from crosses of Marquis with Yaroslav *emmer*. He produced two wheats, Hope and H44, with 42 chromosomes that in the mature-plant stage are highly resistant to stem rust. Neither of these wheats is entirely satisfactory in other characters. The resistance of Hope and H44 to all rust races in the field under normal conditions in North America is dependent upon one or two major genetic factors for resistance. Most of the more promising new spring wheats have Hope or H44 somewhere in their parentage.

Corn Breeding.—The viewpoint has been expressed by various writers that the production of adapted corn hybrids for different regions of the corn belt will have the most far-reaching effect of any phase of work in crop improvement of the present generation. The hybrid Burr-Leaming was first distributed in Connecticut in 1922, but the acreage grown of this hybrid has been very small. The first distribution of hybrids in the corn belt occurred from 1932 to 1934, and in 1938 and 1939 from 15 to 25 million acres were planted to hybrid corn, leading to an increased production

of from 100 to 150 million bu. of corn over what would have been obtained if hybrid corn had not been available. Many agronomists believe that there will continue to be a rapid increase in the use of hybrid corn in the years to come until the greater part of the acreage of corn in the United States is planted to hybrid varieties.

The widespread interest in hybrid corn is due primarily to the superiority of hybrids over normal varieties in a number of characters. Although higher yields per acre are important, other improvements are of equal and perhaps greater value. Ability to withstand lodging and resistance to smut and to ear and stalk rots are of major importance. The development of drought-resistant and frost-resistant hybrids has been studied also, although much remains to be accomplished in these fields.

In the development of hybrid corn, Mendelian principles have been used directly. A standardized technic of breeding has been developed based on the direct application of principles of genetics.

Intensive studies of inbreeding and crossbreeding corn were started by E. M. East at the Connecticut Agricultural Experiment Station and G. H. Shull at Cold Spring Harbor in 1905. Many investigators have taken part in studies of inheritance in maize. The fundamental principles elucidated have led to a sound basis for scientific improvement in corn, a field in which a considerable number of investigators devote all or part of their research efforts. Some of the more important principles leading to the present methods will be mentioned, although corn breeding will be outlined in detail in a later chapter.

1. Continued self-pollination in corn leads to the production of relatively homozygous types that are in general less vigorous than normal corn. Crossing inbreds restores vigor. Some F_1 crosses are more vigorous than normal corn; others, less so.

2. Crosses between inbreds are difficult to use in commercial seed production, since the yield of seed per acre is low. This difficulty has been overcome by using for commercial production crosses between single crosses.

3. Hybrid vigor in corn and in other crop plants has been placed on a definite Mendelian basis. It is a result of partially dominant growth factors. Many genes are involved in growth vigor, and consequently linkage makes it difficult to combine all important genes in a single inbred line.

4. Some inbred lines have much better combining ability than others when tested in comparable crosses. By crossing a group of inbreds to be used in a definite breeding program with a variety and by testing the inbred-variety crosses in yield trials, the better combining inbreds can be isolated and the less desirable discarded.

5. The combining value of inbred lines in a double cross can be predicted from yield trials of the appropriate single crosses. From each of four inbred lines, six single crosses and three double crosses can be made. Yields of any particular double cross can be predicted from the average yield of the four single crosses not used in making the double cross. These results may be understood on the basis that a double cross produced from advanced generations of two single crosses behaves approximately the same as the double cross between the two single crosses.

6. The ease of commercial production of double-crossed seed is dependent to a considerable extent upon the vigor of the inbred lines as well as the yielding ability of the single crosses used in the double cross. Improved inbred lines of corn can be bred by the same breeding methods as used in the production of improved varieties of self-pollinated plants, although it is necessary to control pollination by appropriate selfing and crossing in carrying out the program.

7. The principles of corn breeding that make possible the utilization of hybrid vigor are dependent upon an understanding of genetic principles and their application to corn breeding. This knowledge has made possible to a considerable extent the standardization of corn-breeding technics.

Potato Improvement.—Since commercial varieties of potatoes are highly heterozygous, plants grown from seed will vary greatly. The selection and clonal increase of plants developed from seed was naturally the first breeding method used and led to the production of the old standard varieties. During the early period of the present century, selection within clones was used as a method of potato improvement. Although of little value in breeding, clonal selection was an aid in isolating plants free from virus diseases. This led to the use of the tuber-unit method in the diagnosis for and eliminating of plants affected by virus diseases.

Most varieties of the potato are nonself-fruitful, but strains have been isolated that are highly self-fruitful. Clones of the

latter type produce an appreciable amount of stainable pollen. The partial pollen sterility found in the self-fruitful clones appears to be due to abortion of the pollen grains after regular meiotic division. In the nonself-fruitful clones very little stainable pollen is produced because of irregular meiotic division. In crosses between self- and nonself-fruitful clones, the progeny are usually highly nonself-fruitful.

Improvement of self-fruitful clones may be accomplished through selfing and selection within clones and crosses between clones, with the use of the breeding methods applicable to self-fertile crops. By the use of such methods clones resistant to late blight, scab, and specific virus diseases have been produced. The breeding value of selections in self-fruitful clones may be determined by tests of clonal progenies or, preferably, by tests of the selfed progeny. Thus, two clones may appear to produce the same amount of disease but be found to differ in genotype when selfed progenies of these clones are compared. The genotype of clones that are pollen-sterile may be determined from crosses with pollen-fertile clones of known genotypes by the use of the pollen-sterile clones as females.

The use of inbreeding methods, supplemented by planned crosses, has resulted in a rapid increase in knowledge of the genetics of the potato and the isolation of superior germ plasm in this crop. In the utilization of this superior germ plasm, some modifications in the ordinary breeding, however, must be made in the methods applicable to self-fertile crops.

Although self-fertility is of value in the isolation and synthesis of strains that are resistant to disease or insect attack and have desirable agronomic characters, self-fruitfulness in itself leads to a loss in yield of tubers. Plants that produce flowers and fruits yield less than plants that do not, the reduction in yield being proportional to the number of flowers or fruits produced. Consequently, the character of self-fruitfulness, after being utilized during the production of superior strains, must be eliminated in the breeding of commercial varieties. This may be accomplished through crosses between nonself-fruitful commercial varieties possessing high yielding capacity and certain plant and tuber types with self-fruitful clones that possess the characters to be added, with the use of the self-fruitful clones as pollen parents. The F_1 progeny of such crosses are highly nonself-fruitful.

Selection of nonfruiting types in these progenies may be expected to result in improved varieties into which have been synthesized the characters desired.

The use of such methods of breeding has resulted in the development and release to the growers of more than a dozen new varieties during the past six years. All these have as one parent at least a superior pollen parent developed at the Minnesota Agricultural Experiment Station or by the U. S. Department of Agriculture. Some of these varieties are resistant to late blight; others are resistant to specific viruses, common scab, or insect attack. Warba is a newly developed early maturing variety that is resistant to mosaic and possesses high-yielding ability. The Sebago variety withstood the severe late blight epidemic of 1938 remarkably well. Katahdin, with its viable pollen, provides the plant breeder with a high-yielding commercial variety that can be used as a pollen parent in further breeding. The synthesis of commercial varieties that have resistance to several diseases, as well as many desirable agronomic characters, is preceeding rapidly.

CHAPTER II

THE GENETIC AND CYTOGENETIC BASIS OF PLANT BREEDING

A knowledge of the chromosome basis of heredity is essential to the breeder. The characters of a plant are the end result of the interaction of genes, carried in the chromosomes, under particular environmental conditions. What is inherited is the manner of reaction and not the character itself.

Diploid organisms result from the union of male with female reproductive cells, the chromosome number in the zygote normally being twice that of the gamete. With self-pollinated organisms, homozygosis is obtained automatically, and permanence of characters under uniform conditions of environment is obtained as a result of equational division of each chromosome and gene during somatic mitosis, the diploid conditions of the chromosomes in the body and the pairing during reduction division of like chromosomes, two by two, leading to the production of a single genetic type of gamete.

The linear arrangement of genes in the chromosome has been generally accepted, and the division of the gene in mitosis and its segregation in meiosis have furnished the mechanism for the transfer of the unit of inheritance, the gene, from cell to cell. A knowledge of the number and nature of the chromosomes in each crop plant and their behavior in cell division is fundamental to the study of plant breeding. Mendel contributed the law of independent inheritance, and Bateson and Punnett in 1906 gave the first case of linkage in sweet peas in a cross between a purple-flowered variety with long pollen and a red-flowered variety with round pollen. The phenotypic condition in the backcross of 50 purple long, 7 purple round, 8 red long, and 47 red round plants was explained on the basis of gametic production in the ratio of 7 purple long, 1 purple round, 1 red long, and 7 red round instead of by the usual gametic ratio of 1:1:1:1. The parental combinations were formed seven times as frequently as the new combinations.

TABLE 1.—CHROMOSOME NUMBER (HAPLOID) IN THE COMMON CROP PLANTS

Scientific name	Common name	Number of chromosomes (*n*)
Cereal Crop Plants and Relatives		
	Wheat:	
Triticum monococcum	Einkorn	7
Triticum dicoccum	Emmer	14
Triticum durum	Durum	14
Triticum spelta	Speltz	21
Triticum vulgare	Bread	21
	Oats:	
Avena brevis	....	7
Avena strigosa	....	7
Avena barbata	.....	14
Avena fatua	Wild	21
Avena sativa	Cultivated	21
Avena byzantina	Red cultivated	21
Avena nuda	Hull-less	21
	Barley:	
Hordeum distichon	2-row barley	7
Hordeum deficiens	2-row barley	7
Hordeum vulgare	6-row barley	7
Hordeum jubatum	Squirrel tail	14
Hordeum nodosum	.....	21
Secale cereale	Rye	7
Fagopyrum esculentum	Buckwheat	8
Oryza sativa	Rice	12
Zea mays	Corn	10
Sorghum halepensis	Johnson grass	20
Sorghum vulgare	Milo, Kafir, Feterita, Kaoliang	10
Sorghum vulgare, var. *sudanensis*	Sudan grass	10
Forage Grasses		
Agropyron cristatum	Crested wheat	7, 14
Agropyron pauciflorum	Slender wheat	14
Agrostis alba	Red top	21
Alopecurus pratensis	Meadow foxtail	14
Andropogon furcatus	Big bluestem	35
Andropogon scoparius	Little bluestem	20
Bromus inermis	Brome grass	21, 28
Dactylis glomerata	Orchard grass	14
Elymus canadensis	Wild rye	14
Festuca elatior	Meadow fescue	7, 14, 21, 35
Lolium italicum	Italian rye grass	7

TABLE 1.—CHROMOSOME NUMBER (HAPLOID) IN THE COMMON CROP PLANTS.—(*Continued*)

Scientific name	Common name	Number of chromosomes (*n*)
Forage Grasses—(*Continued*)		
Lolium perenne	Perennial rye grass	7
Panicum miliaceum	Proso millet	18, 21
Phalaris arundinaceae	Reed canary	7, 14
Phleum pratense	Timothy (American)	21
Phleum pratense	Timothy (British)	7
Poa compressa	Canada blue	7, 21, 28
Poa pratensis	Kentucky blue	14–49 ±
Legumes		
Glycine soja	Soybean	20
Lespedeza sp	Japan clover	9, 10, 18
Medicago falcata	Alfalfa	8, 16
Medicago sativa	Alfalfa	16
Melilotus alba	Sweet clover (white)	8
Melilotus officinalis	Sweet clover (yellow)	8
Pisum sativum	Pea	7
Trifolium hybridum	Alsike clover	8
Trifolium pratense	Red clover	7, 12
Trifolium repens	White Dutch clover	8, 12, 14, 16
Vigna sinensis	Cowpea	12
Fiber Plants		
Cannabis sativa	Hemp	10
Gossypium sp	Cotton (Asiatic)	13
Gossypium sp	Cotton (American)	26
Linum usitatissimum	Flax	15, 16
Sugar Plants		
Beta vulgaris	Sugar beet	9
Saccharum officinarum	Sugar cane	40–63
Stimulants		
Coffea sp	Coffee	11, 22, 33, 44
Nicotiana tabacum	Tobacco	24
Thea sinensis	Tea	12-13, 15, 22–23
Oil Plants		
Aleurites sp	Tung oil	11
Arachis hypogaea	Peanut	10, 20
Linum usitatissimum	Flax	15, 16
Sesamum indicum	Sesame	26

TABLE 1.—CHROMOSOME NUMBER (HAPLOID) IN THE COMMON CROP PLANTS.—(*Continued*)

Scientific name	Common name	Number of chromosomes (*n*)
	Vegetables	
Allium cepa	Onion	8
Asparagus officinalis	Asparagus	10
Beta vulgaris	Beet	9
Beta vulgaris var. *cicla*	Chard	9
Brassica oleracea	Cabbage, cauliflower, kohlrabi	9
Brassica rapa	Turnip	10
Capsicum annuum	Pepper	12
Citrullus vulgaris	Watermelon	11
Cucumis melo	Muskmelon	12
Cucumis sativus	Cucumber	7
Cucurbita moschata	Squash	20
Cucurbita pepo	Pumpkin	20
Lactuca sativa	Lettuce	9
Lycopersicum esculentum	Tomato	12
Phaseolus lunatus	Bean (lima)	11
Phaseolus vulgaris	Bean (kidney)	11
Pisum sativum	Pea	7
Raphanus sativus	Radish	9
Rheum rhaponticum	Rhubarb	22
Solanum melongena	Eggplant	12
Solanum tuberosum	Potato	24
Spinacia oleracea	Spinach	6
	Fruits	
Citrus grandis	Grapefruit	9
Citrus limonia	Lemon	9
Citrus sinensis	Common orange	9, 18
Fragaria grandiflora	Strawberry (cultivated)	28
Malus malus	Apple	17, 51½
Prunus americana	Plum (American)	8
Prunus domestica	Plum (European)	24
Prunus avium	Cherry (sweet)	8
Prunus cerasus	Cherry (sour)	16
Prunus persica	Peach	8
Pyrus communis	Pear	17, 51½
Ribes sp	Currant	8
Rubus idaeus	Red raspberry (European)	7, 14
Rubus strigosus	Red raspberry (American)	7
Vitis sp	Grape (cultivated)	19, 20, 38

The frequencies of new combinations of factor pairs lying in homologous chromosomes are dependent to a great extent upon the distance apart of the genes in the chromosome. The spindle fiber apparently reduces crossing over in adjacent regions. There is no interference between crossovers on opposite sides of the spindle fiber. Many studies of genetic linkages have shown wide differences between genetic maps and physical-map distances as determined by cytogenetic study of induced breaks in chromosomes. These studies have given added evidence, however, of the linear order of the genes in the chromosome. There are also other types of cytological aberrations, such as inversions and translocations that lead to new genetic maps. In crosses between the new types with standard types, genetic ratios may be greatly modified.

Qualitative and quantitative differences in chromosomes have been extensively studied in recent years, and the field of cytogenetics is being constantly developed. Information regarding the number and nature of chromosome differences is being obtained rather rapidly for many crop plants. The present chapter will summarize the usual chromosome numbers in important crop plants and illustrate how genetic and cytogenetic principles are being used by the plant breeder. Chromosome numbers in the common crop plants, taken largely from the 1936 and 1937 *U. S. Department of Agriculture Yearbooks*, are given in Table 1.

POLYPLOIDS IN RELATION TO PLANT BREEDING

A study of chromosome numbers in related species of economic plants shows many multiple series. A common haploid number in the *Gramineae* is 7. Species of *Triticum*, *Avena*, and *Hordeum* with haploid numbers of 7, 14, and 21 are common and illustrate a type of variation in polyploids, *i.e.*, multiples of a fundamental number, often referred to as a euploid series. Aneuploidy, or variation in chromosome numbers not multiples of a fundamental number, is frequent in some species. *Poa pratensis*, for example, varies from 28 to over 100 somatic chromosomes. Aneuploid chromosome numbers are of more frequent occurrence in species that have apomictic development than under conditions of sexual reproduction.

There are two main types of euploids, namely, allopolyploids and autopolyploids. These two types are illustrated in Fig. 2. In this illustration the autopolyploid is of the autotetraploid type and has four sets of like or homologous chromosomes. There may be random pairing or mating between each group of the four homologous chromosomes. An allopolyploid has chromosome sets from different sources. In the illustration a haploid set from

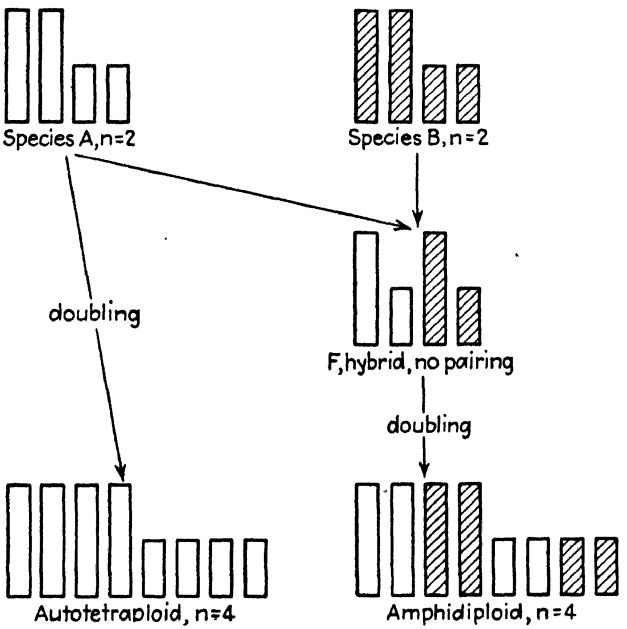


FIG. 2.—Schematic diagram of development of autotraploid due to doubling of chromosome number in the zygote and the production of an amphidiploid from a cross between related species due to doubling of chromosome number in the gametes or zygotes when chromosomes are so unlike that pairing is not obtained in the F_1 cross.

species *A* is so different from that of species *B* that pairing does not take place. Doubling of the chromosome number will result in an allopolyploid of the amphidiploid type.

One of the best known cases of triplicate factors in a polyploid of the amphidiploid type is in *Triticum vulgare*, where there may be three pairs of factors for brownish red color of the kernel, any one of the three in a dominant condition leading to the development of color. This case was given originally by Nilsson-Ehle as the basis for the multiple-factor theory of quantitative inherit-

ance, without a knowledge of the fact that *T. vulgare* is a polyploid of the hexaploid type with the amphidiploid type of chromosome pairing, *i.e.*, contains three sets or genoms of seven bivalents (7_{II}) each, or 42 somatic chromosomes. These three factor pairs for red kernel color may be designated R_1r_1, R_2r_2, R_3r_3. An illustration of the mode of inheritance of red vs. colorless kernels may be given where only two of the three factor pairs are concerned and the parents and F_1 produced red kernels.

	Variety A		*Variety B*
Parental phenotype	red		red
Parental genotype	$R_1R_1\ r_2r_2\ r_3r_3$		$r_1r_1\ R_2R_2\ r_3r_3$
F_1 phenotype		red	
F_1 genotype		$R_1r_1\ R_2r_2\ r_3r_3$	

Since the r_3r_3 factor pair is in the homozygous recessive condition, it will have no effect on kernel color and may be disregarded.

The following summary gives the genotype and kernel color of F_2 plants and F_3 breeding behavior:

F_2		F_3
Genotype	Kernel color	Breeding behavior
1 $R_1R_1R_2R_2$	Red	Breeds true for red kernel color
2 $R_1r_1R_2R_2$	Red	Breeds true for red kernel color
2 $R_1R_1R_2r_2$	Red	Breeds true for red kernel color
4 $R_1r_1R_2r_2$	Red	Segregates; 15 plants red kernels: 1 plant colorless
1 $R_1R_1r_2r_2$	Red	Breeds true for red kernel color
2 $R_1r_1r_2r_2$	Red	Segregates; 3 plants red: 1 plant colorless
1 $r_1r_1R_2R_2$	Red	Breeds true for red kernel color
2 $r_1r_1R_2r_2$	Red	Segregates; 3 plants red: 1 plant colorless
1 $r_1r_1r_2r_2$	Colorless	Breeds true for colorless kernels

Genotypes $R_1r_1R_2R_2$ and $R_1R_1R_2r_2$ are illustrations of polyploids with genetic segregation that has no definite phenotypic effect. There is a general relation between intensity of color and number of dominant factors, but the relation is so indefinite that the number cannot be estimated by visual inspection.

The segregation in F_2 of 15 plants with red kernel color to 1 with colorless kernels results from crossing two varieties, the one homozygous for $R_1R_1r_2r_2$ and the other $r_1r_1R_2R_2$. Of the 15 plants with red kernel color in F_2, 7 breed true in F_3 for red color,

4 segregate in a 15:1 ratio, and 4 segregate in a 3:1 ratio. The plants with colorless kernels in F_2 breed true for this color in F_3.

Contrasted with the foregoing allopolyploid type is the autopolyploid, such as is obtained in *Datura stramonium* resulting from doubling of the diploid chromosome number. The chromosome constitution may consist of four identical sets of chromosomes. If we take the case where the diploid was heterozygous for a single factor pair *Dd*, the autotetraploid will have the genotype *DDdd*. Such a polyploid has a high frequency of quadrivalent association, and random chiasma formation occurs among the four homologous chromosomes.

In an autotetraploid for any dominant factor, there may be a series of genotypes such as *DDDD*, *DDDd*, *DDdd*, *Dddd*, *dddd*, also written D_4, D_3d, D_2d_2, etc., respectively. In an autopolyploid there may be chromosome segregation or random chromatid segregation. Random chromatid segregation occurs only when the factors concerned are somewhat more than 50 crossover units from the spindle-fiber attachment. For closer distances the ratios are intermediate between those expected from chromosome and chromatid segregation, approaching chromosome segregation as the genes become closer to the spindle fiber. This naturally modifies the ratios obtained from particular types of heterozygotes.

A convenient method of calculating gametic expectation is illustrated as follows in an autotetraploid of the D_3d (*DDDd*) type. The number of combinations of n things taken r at a time $= \frac{n!}{(n-r)!r!}$.

For chromosome segregation, the gametic expectation may be calculated in the following manner. Two types of gametes would be obtained, *DD* and *Dd*. The gametic ratio expected is as follows:

For gamete *DD*, or the number of different ways of taking two things out of three, $n = 3$ and $r = 2$, and $\frac{3!}{1!2!} = 3DD$.

For diploid gametes containing a dominant and recessive factor, *Dd*, for example, it is unnecessary to use the formula. The *D* factor can be taken in three ways from D_3, whereas *d* can be taken in only one. The gametic expectation then will be $3D \times 1d = 3Dd$. This is the result that would be obtained if the

formula was used to calculate the number of combinations and if it is remembered that factorial zero (0!) equals 1.

With random chromatid segregation, however, the condition would be entirely different. The chromatid condition would be D_6d_2.

The number of different ways of obtaining a combination of *DD* can be calculated by substituting, where $n = 6$ and $r = 2$ in the formula. The frequency of gametes of the *DD* type will be $\frac{6!}{4!2!} = \frac{2 \cdot 3 \cdot 4 \cdot 5 \cdot 6}{2 \cdot 3 \cdot 4 \cdot 2} = 15DD$. Gametes of the *Dd* type can be obtained by finding how many times one *D* can be taken from D_6, which equals $6D$, and this multiplied by $2d$ would give $12Dd$. One gamete of the *dd* type can be obtained, and the gametic ratio then will be $15DD:12Dd:1dd$. Thus, from selfing *DDDd*, where the percentage recombination between the *D* locus and the spindle fiber approaches 50 per cent, the phenotypic expectation in F_2 is $783D:1d$. Such peculiar ratios cannot easily be differentiated from mutations without studying second-generation selfed progeny rather extensively.

In a similar manner, the student can calculate expectation for other genetic types of polyploids. Linkage relations are greatly complicated in polyploids. The student of plant breeding will be able to determine logical explanations for the results obtained only when a knowledge is available of the chromosome mechanism for the particular plant under study.

There are two general types of euploids, but probably, in many cases, there are also intermediates that behave as amphidiploids in some cases and for some chromosome pairs and autopolyploids under other conditions or for other chromosome pairs. Wheat is a good example of a hexaploid that generally gives an amphidiploid type of inheritance. Ordinary bread wheat contains three sets, or genoms, of 7 chromosomes each, and, as a rule, the pairing behavior is of the diploid type. Based upon Winge's original explanation, doubling could occur from crosses between two closely related species each with a chromosome number of $n = 7$, which, through geographical isolation, gene mutations, and chromosomal changes, had become so differentiated in their chromosome mechanism that crossing was possible but pairing did not occur in meiosis. This may have led to the inclusion of 28 chromosomes in a single cell, resulting from equational division

of 14 unpaired chromosomes, 7 being obtained from each parental species. A further cross between this species with another closely related form with $n = 7$ chromosomes would furnish the type basis for an amphidiploid form with $n = 21$ chromosomes. Doubling has occurred in experimental material of this nature both in the zygote and in the gamete.

There are many illustrations in the literature of pairing of the autopolyploid type in a polyploid that usually pairs as an amphidiploid. Breeders of crop plants with the amphidiploid-chromosome condition obtain variations in breeding behavior that seem most logically explained on the basis of changes in the type of chromosome pairing.

Genom analyses based on types of chromosome pairing have been made extensively with *Triticum* and related genera by numerous investigators. One of the first summaries was that given by Gaines and Aase (1926), illustrated by Fig. 3.

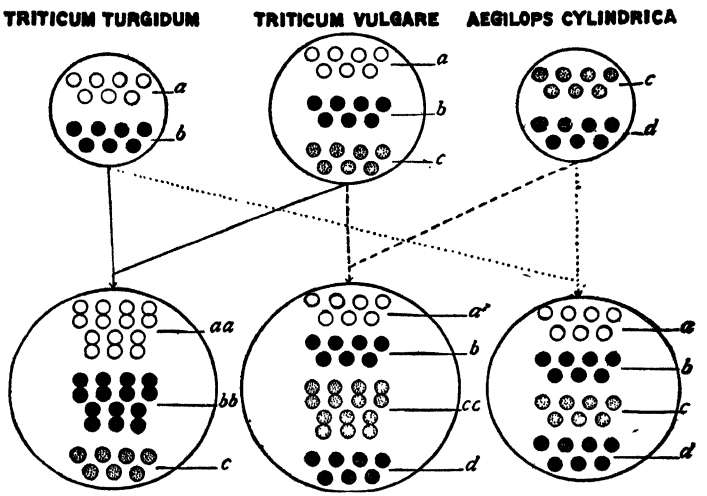


FIG. 3.—Diagram illustrating hypothetical relationships of chromosomes. The 7 chromosomes in set *a* and the 7 chromosomes in set *b* are present in both *Triticum vulgare* (21 chromosomes as the haploid number) and in *T. turgidum* (14 chromosomes). The 7 chromosomes in set *c* are present in *T. vulgare* and in *Aegilops cylindrica* but not in *Triticum turgidum*. The 7 chromosomes in set *d* are present in *Aegilops* but not in either *T. vulgare* or *T. turgidum*. A 21-chromosome wheat × a 14-chromosome wheat gives rise to sporocytes with 14 paired and 7 unpaired chromosomes (lower left). A 21-chromosome wheat × *Aegilops cylindrica* gives rise to sporocytes with 7 paired and 21 unpaired chromosomes (lower center). *Aegilops cylindrica* × *Triticum turgidum* gives rise to sporocytes with 28 unpaired chromosomes (lower right).

The present status of the problem may be summarized as follows:

EINKORN SERIES
($n = 7$)
AA
Triticum aegilopoides
Triticum monococcum

TIMOPHEEVI SERIES
($n = 14$)
AAGG
Triticum timopheevi

AEGILOPS SERIES
($n = 14$)
CCDD
Aegilops cylindrica

EMMER SERIES
($n = 14$)
AABB
Triticum dicoccoides
Triticum dicoccum
Triticum durum
Triticum turgidum
Triticum pyramidale
Triticum polonicum
Triticum persicum

SPELT SERIES
($n = 21$)
AABBCC
Triticum spelta
Triticum vulgare
Triticum compactum

SECALE SERIES
($n = 7$)
EE
Secale cereale

The species of *Triticum*, *Secale*, and *Aegilops* are seen to be made up of one or more sets (genoms) of seven chromosomes each, designated *A*, *B*, *C*, *D*, *E*, and *G* (Lilienfeld and Kihara 1934, Kostoff 1937). In crosses between the Emmer and Spelt series, for example, the pairing behavior in F_1 most commonly consists of 14_{II} and 7_I, although in some cases a few trivalents and quadrivalents may be obtained because of the fact that chromosomes of one genom have some homology with those of a different genom. One of the genoms of *timopheevi* is similar to the *A* genom, the other (*GG*) resembles *B* more closely than *C* but differs rather widely from *B*, forming from two to seven loose conjugations with it.

Stadler (1928, 1929) studied rate of induced mutation per *r* unit in barley, oats, and wheat in relation to chromosome numbers. Results are as follows:

Species	Number of chromosomes (n)	Rate of mutation
Hordeum vulgare	7	4.9 ± 0.9
Avena brevis	7	4.1 ± 1.2
Avena strigosa	14	2.6 ± 0.6
Avena sativa	21	0
Triticum monococcum	7	10.4 ± 3.4
Triticum dicoccum	14	2.0 ± 1.3
Triticum durum	14	1.9 ± 0.5
Triticum vulgare	21	0

In general, as has been mentioned, there may be three sets of factors in hexaploid wheat and oats but only a single factor pair in each locus for diploid species. A mutation in a homozygous form *AA* in barley, giving *Aa*, would produce progeny containing 25 per cent of recessives. In a tetraploid of the amphidiploid type, two such simultaneous mutations would be necessary in order than an induced mutation for a character that was doubly dominant could show up in the immediate progeny.

Variation in pairing whereby one chromosome pair of a genom shows some homology with a member of a different set would lead to abnormal segregation. In polyploids of the amphidiploid type, such results are probably of relatively frequent occurrence.

Powers (1932) and Myers and Powers (1938) have studied variability in strains of wheat due to various types of chromosome abnormalities or to gene differences. In a study of Marquillo, a variety belonging to the spelt series with a haploid chromosome complement of 21 but derived from a cross between varieties of *Triticum durum* and *T. vulgare* with 14 and 21 haploid chromosomes, respectively, Powers (1932) found that germinal instability in Marquillo was greater than in Marquis or Thatcher. Thatcher is a variety produced by crossing a sister selection of Marquillo with a purified hybrid of Marquis × Kanred.

In these studies, the easiest method of estimating the percentage of germinal instability was to measure the occurrence of chromatin loss, measured by the frequency of occurrence of microspores showing micronuclei. The mean percentage of micronuclei in four varieties is given in the following summary, taken from Myers and Powers:

Variety	Total plants	Mean percentage of micronuclei
Thatcher	25	0.8
Marquis	26	0.9
H44	20	4.1
Supreme	9	8.3

H44 is a variety of wheat produced by McFadden from a cross of Yaroslav *emmer* × Marquis. It has the chromosome number of the spelt group. Supreme is a variety of *T. vulgare* produced by selection from Red Robs.

Although it seems probable that a wheat of recent origin such as Marquillo may show greater germinal instability than old established varieties such as Marquis, as has been pointed out by Powers and also by Love (1938), it seems of interest that Supreme, a variety of *T. vulgare*, selected from a variety not of recent origin, is equally instable, although there is the possibility that its origin may be also the result of a natural cross between species. Myers and Powers showed germinal instability to be inherited, and the isolation of apparently homozygous lines with different percentages of micronuclei was considered to indicate that genetic factors were involved in conditioning meiotic instability.

In the studies of Marquillo, Powers found evidence for 7.2 per cent of natural crossing. This is higher than has been usually observed with other varieties of wheat at the Minnesota station. Thirty-two plants of Marquillo were studied, two of these having only 41 chromosomes. An average of 23.4 $\pm$ 0.24 per cent of the microspores of the 41 chromosome plants showed micronuclei, whereas only 2.8 $\pm$ 0.16 per cent of the 42 chromosome plants showed micronuclei.

In a recent cross in oats by Hayes, Moore, and Stakman (1939) between Bond, *Avena byzantina*, and standard varieties of *A. sativa*, segregation in F_2 for type of base on the lower floret occurred in a 3:1 ratio of sativa to byzantina types. Several F_3 families from F_2 plants showed wide deviations from the type of segregation in F_2 and an intermediate type of base bred true in later generations. The hypothesis that these results were due to change in chromosome pairing was used, although further studies are necessary to prove the hypothesis.

Hope and H44 are vulgare wheats with $n = 21$ chromosomes that descended from crosses of *Triticum dicoccum* $\times$ Marquis (*T. vulgare*). In many studies of stem-rust reaction, when Hope and H44 are crossed with other varieties of vulgare wheats, wide deviations from the usual type of F_2 segregation have been observed in F_3 families. In such crosses, however, it has been relatively easy to obtain homozygous types with stem-rust resistance similar to that of the Hope and H44 parents. Changes in the manner of chromosome pairing in complex polyploids of the amphidiploid type seem to occur rather frequently. This tends to complicate breeding behavior and necessitates that greater care be used to ensure the selections of greatest promise

are breeding true before they are increased for distribution. A tendency for a modified type of segregation in F_3 in some families does not necessarily greatly complicate the breeder's problem of selecting desirable homozygous types.

Speltoid wheats and fatuoid oats have occurred in *T. vulgare* ($2n = 42$) and *A. sativa* ($2n = 42$) as a result of chromosomal variations due very probably to a change in chromosome pairing. The review of Sansome and Philp (1939) has been used in this summary. Speltoid wheats resemble *T. spelta* and fatuoid, or false wild oats, resemble *A. fatua.* Three types have been observed: Type *A*, with no change in chromosome numbers, Type *B*, with a chromosome deficiency, and Type *C*, with a chromosome excess.

The *A* type, when heterozygous, gives three types of progeny: homozygous fatuoid or speltoid, heterozygous, and normals in a ratio of 1:2:1.

An explanation that has been given by Winge and Huskins is on the basis of a change in pairing due to the similarity of a chromosome of one genom with that of another. If we designate the three chromosome pairs concerned, as *A*, *B*, and *C*, one chromosome belonging to each of the three genoms, and suppose the *B* chromosome carries the speltoid factors and *C* the normal factors epistatic to the speltoid, then the normal type $\frac{ABC}{ABC}$ would breed true, as a rule, for absence of speltoid characters. If one supposes that *B* has sufficient homology with *C*, so that occasional pairing occurs between *B* and *C*, giving rise to $\frac{ABB}{ACC}$, then gametes *ABB* and *ACC* would be obtained. If gamete *ABB* mated with *ABC*, the heterozygous speltoid form $\frac{ABC}{ABB}$ would be obtained. On selfing, three types of progeny would result—normals, $\frac{ABC}{ABC}$, heterozygous speltoids, $\frac{ABC}{ABB}$, and homozygous speltoids, $\frac{ABB}{ABB}$, in a ratio of 1:2:1. Such a homozygous speltoid would give some quadrivalent associations, as was observed by Winge, whereas the heterozygous speltoid would show trivalent and univalent associations that were observed

also. The old hypothesis that fatuoids arise through natural crosses between *Avena sativa* and *A. fatua* is seen to be untenable.

Winge gave formulas for the *B* and *C* types of speltoids where *O* stands for the loss of a chromosome, consisting of the heterozygous type $\frac{ABO}{ABC}$ and two sorts of homozygotes, one $\frac{ABO}{ABB}$, with the loss of a chromosome, and the other $\frac{ABBB}{ABB}$, with the duplication of the chromosome *B*.

Huskins studied breeding behavior, variations in pairing, and chromosome numbers in fatuoids or false wild oats, obtaining the same sort of results that have been outlined for speltoids.

These types of results have been presented briefly to emphasize the difficulties of studying genetics in polyploids. The plant breeder must deal with polyploids in economic plants, and a knowledge of the causes of variability may aid greatly in the breeding program. Selection for types with chromosome pairing that will give normal disjunction will aid in establishing uniform breeding strains. Wide deviations from normal types of segregation may be expected in some progenies. There is, however, considerable evidence that by selection for germinal stability, in many cases, the variability resulting from abnormal pairing may be overcome. Such selection will often be of great economic importance, since germinal instability often leads to the production of undesirable characters. The loss or gain of one or more chromosomes in polyploids may lead to the production of an undesirable type of abnormality, such as speltoid wheat or fatuoid oats.

SOME APPLICATIONS OF GENETICS TO PLANT BREEDING

The value to the plant breeder of a knowledge of the mode of inheritance of important characters and the application of genetic principles to methods of breeding will be illustrated by specific examples. A problem in oat improvement recently investigated at the Minnesota Experiment Station illustrates the use of genetics in a practical breeding problem. The parent varieties and character differences are summarized here.

Anthony, Iogold, Rainbow	Bond
1. Good yield*	Fair yield
2. Good-quality grain	Excellent quality grain*
3. Fair straw strength	Excellent standing ability*
4. Stem-rust resistance*	Stem-rust susceptibility
5. Susceptibility to crown rust	Crown-rust resistance*
6. Susceptibility to smuts	Smut resistance*
7. Sativa type*	Byzantina type

* Characters desired in the hybrid.

Frequently certain crosses give a larger proportion of desirable offspring than others, probably because of the fact that the genotype of the one parent supplements that of the other in a more satisfactory manner, although the reason why certain crosses give a greater proportion of desirable progeny than others, in many cases, cannot be placed on a definite genetic basis. These facts have led the plant breeder to use several crosses for a specific problem rather than a single cross.

Anthony, Iogold, and Rainbow were three recommended varieties of *Avena sativa* grown by Minnesota farmers. Iogold, because of early maturity, is adapted to southern Minnesota; the midseason varieties Antony and Rainbow usually yield better than Iogold in north central and northern Minnesota. Double Cross A, also crossed with Bond, was a selection from (Minota × White Russian) × Black Mesdag, homozygous resistant for the White Russian type of stem-rust reaction and the Black Mesdag type of resistance to smut. Although not particularly desirable in type of kernel, it was outstanding in yielding ability.

Anthony, Iogold, and Rainbow were selected because they produced good yields of grain, were resistant to stem rust, caused by *Puccinia graminis avenae* Eriks. & Henn., and were of the sativa type. Cultivated varieties of *Avena sativa* have proved more desirable in Minnesota. Bond, a variety of *A. byzantina*, is highly resistant to crown rust, *Puccinia coronata* Corda, and to the smuts prevalent in Minnesota, *Ustilago avenae* (Pers.) Jens. and *Ustilago levis* (Kellermann & Swingle) Magn. Bond excels also in ability to withstand lodging and in grain quality, producing plumper kernels with a higher weight per bushel than the recommended varieties of *A. sativa*.

A summary of the mode of inheritance of the characters will help to explain the way that genetic principles can be used in a breeding program.

MODE OF INHERITANCE OF CHARACTERS IN OAT CROSSES
(ANTHONY, IOGOLD, RAINBOW × BOND)

1. Crown rust. F_1 resistant; F_2 9 resistant:7 susceptible or 3 resistant:1 susceptible.
2. Stem rust. F_1 resistant; F_2 3 resistant:1 susceptible.
3. Smuts. Using a mixture of races of the two smut species, F_1 resistant; F_2 segregation 1 to 3 pairs of genes.
4. Sativa vs. byzantina characters.
   a. Spikelet disarticulation. F_1 sativa base; F_2 3 sativa base:1 byzantina base.
   b. Floret disjunction. F_1 byzantina type; F_2 1 or 2 pairs of factors.
   c. Basal hairs. F_1 sativa type; F_2 3 sativa:1 byzantina.
5. Yield. Multiple factors.
6. Time of maturity. Multiple factors.
7. Plumpness of grain. Multiple factors.

The purpose of the crosses was to combine in a single variety the desirable agronomic characters with resistance to three major oat diseases, stem rust, crown rust, and smuts. In these studies the pedigree method of breeding was used. It consisted of growing the segregating generations as progenies so that individual plant study was possible, each progency consisting of approximately 50 plants from a single plant of the previous generation. Selection was continued until homozygous lines were obtained that appeared desirable. Then the best lines were determined from replicated yield trials.

Crown Rust.—In the cross of Rainbow × Bond, only a single factor pair was involved. In F_2 in the other crosses, there were approximately 9 crown-rust-resistant to 7 susceptible plants. The Bond type of resistance appeared to be due to the complementary action of two factors. Resistance to each physiologic race to which Bond was resistant seems to be due to the same genetic factors.

The illustration shown at the top of page 28 is given where two factor pairs were necessary to explain the results. F_2 genotypes and phenotypes and F_3 breeding behavior are summarized.

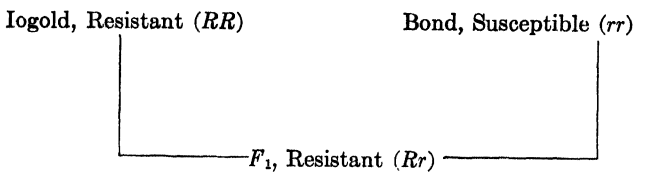
F_2 plants resistant to crown rust were selected. On the average 1 out of 9 may be expected to breed true for resistance in F_3. Two types of segregating progenies are expected in F_3, one segregating on a 3:1 basis and the other on a 9:7 basis.

Progenies breeding true for resistance to crown rust can be determined by seedling inoculation in the greenhouse. By growing from 20 to 30 seedlings from each plant selected and by

F_2 genotype	F_2 phenotype	F_3 breeding behavior
1 *AABB*	Resistant to crown rust	Breeds true for crown-rust resistance
2 *AaBB*	Resistant to crown rust	Segregates, 3 resistant:1 susceptible
2 *AABb*	Resistant to crown rust	Segregates, 3 resistant:1 susceptible
4 *AaBb*	Resistant to crown rust	Segregates, 9 resistant:7 susceptible
1 *AAbb*	Susceptible to crown rust	Breeds true for susceptibility
2 *Aabb*	Susceptible to crown rust	Breeds true for susceptibility
1 *aaBB*	Susceptible to crown rust	Breeds true for susceptibility
2 *aaBb*	Susceptible to crown rust	Breeds true for susceptibility
1 *aabb*	Susceptible to crown rust	Breeds true for susceptibility

inoculation with crown rust, the progenies from F_3 to F_5 that are homozygous for crown-rust reaction can be isolated. These breed true for resistance under field conditions.

Stem Rust.—Stem-rust reaction is handled in the same manner. By the use of a single factor pair, the results can be illustrated as follows, where *R* stands for resistance and *r* for susceptibility.



F_2 genotype	F_2 phenotype	F_3 breeding behavior
1 *RR*	Resistant	Breeds true for resistance
2 *Rr*	Resistant	Segregates, 3 resistant:1 susceptible
1 *rr*	Susceptible	Breeds true for susceptibility

As will be discussed in some detail later, many pathogenic organisms are mixtures of races that can be differentiated only by their manner of reaction on a series of varieties used as differential hosts. It has been learned that Iogold and Rainbow are resistant to races of stem rust 1, 2, 3, 5 and 7, that Anthony and Double Cross A are resistant to races 1, 2, and 5, whereas Bond is susceptible to all five races. In this case, there is a series of three alleles

for resistance and susceptibility to stem rust that may be called R_1 for resistance to five races; R_2 for resistance to races 1, 2, and 5; and r for susceptibility to all races.

Crosses of Bond × Anthony or Double Cross A segregate on a 3:1 basis in F_2, and the only two homozygous types that can be obtained will be those that are resistant and susceptible, respectively, to the three races 1, 2, and 5.

Crosses of Bond with Iogold and Rainbow segregate also on a 3:1 basis, in the presence of inoculum of races 3 and 7, whether races 1, 2, and 5 are present or absent, and the two homozygous types that can be obtained will be resistant and susceptible, respectively, to the five races 1, 2, 3, 5, and 7. A consideration of these facts will show that, in crosses of Bond with Anthony, Iogold, Rainbow, or Double Cross A, infection only with race 1 furnishes a satisfactory basis for isolation of the parental type of resistance.

If Anthony or Double Cross A are crossed with Iogold or Rainbow, all offspring are resistant to races 1, 2, and 5, but segregation in F_2 for reaction to races 3 and 7 will occur, giving a 3:1 ratio of resistant to susceptible.

From any cross, therefore, between homozygous members of a multiple allelic series the only homozygous types for the character difference that can be recovered will be the parental types.

There is agreement between seedling and mature-plant reaction for stem-rust and seedling studies can be used in the same general manner for stem-rust reaction as has been outlined for crown rust.

Several races of stem rust can attack the parental varieties Anthony or Iogold, but neither of these varieties has been severely and widely injured by stem rust in farmers' fields under field conditions since their introduction, and Anthony has been grown widely for over 10 years.

Reaction to Smuts.—It was somewhat difficult to determine the genetics of smut reaction. Resistance was dominant over susceptibility, and segregating progenies may be expected to contain fewer susceptible plants than a homozygous susceptible line. Parent rows were included approximately every 20 rows throughout the nursery. In crosses of Bond with Anthony, Iogold, and Rainbow, the results in F_3 indicated a single major-factor pair for smut reaction. In the crosses between Double Cross A with Bond, where both parents were resistant to smut, a few highly

susceptible progenies were obtained in F_3. From previous studies, the resistance of Double Cross A was explained on the basis of two major-factor pairs. The results in the present cross were explained on the basis that the resistance to smuts of the Bond parent was independent in inheritance of the two factors for smut resistance carried by Double Cross A. When all three factor pairs were recessive, susceptibility resulted.

The methods used in selecting for disease-resistant plants were based on a knowledge of the mode of inheritance. An epidemic of crown rust, stem rust, and smut was induced by methods that will be outlined later. Crown rust appears first, and resistant, desirable-appearing plants were selected and tagged about 10 days after heading. Stem rust can be determined at maturity. Plants resistant to stem and crown rust were selected in progenies that were free from smut, and smut-free plants were selected also in progenies that had a lower percentage of smut than the susceptible parents. By these methods it was relatively easy to obtain a large number of progenies resistant to all three diseases.

Sativa vs. Byzantina Characters.—Three pairs of contrasting characters have been used to differentiate byzantina and sativa oats. These may be illustrated by Fig. 4, in which are shown the upper and lower florets of Anthony belonging to *Avena sativa* and Bond, a variety of *A. byzantina.* The characters may be explained briefly.

1. Spikelet disarticulation has been defined as the separation of the lower floret of the oat spikelet from the axis of the spikelet. Three classes were used to describe the segregating generations: (*a*) abscission, typical of Bond, at the right of the figure, leaving a well-defined, deep oval cavity or "sucker mouth" on the face of the callus on the base of the lemma of the lower grain; (*b*) disarticulation by fracture, leaving a rough, fractured surface with little or no cavity at the base of the lemma, characteristic of *A. sativa* as illustrated by Anthony in the figure; and (*c*) disarticulation by semiabscission, intermediate between *a* and *b*.

2. Basal hairs, conspicuous bristles on the base of the lower floret. The Bond parent had long abundant hairs, and the *A. sativa* parents had short hairs that were abundant, few, or absent, depending on the sativa parent used.

3. Floret disjunction, defined as the method of separation of the second floret from the first floret.

The method shown by Bond in Fig. 4, called disjunction by basifracture, is characterized by the rachilla segment breaking near its base and remaining firmly attached to the base of the upper floret, as contrasted with that in Anthony, which has disjunction by disarticulation at the apex of the rachilla segment, the rachilla segment remaining attached to the lower floret. In hybrids, many plants seemed intermediate and were characterized by disjunction by heterofracture, the rachilla segment breaking



FIG. 4.—At left, upper and lower florets of Anthony; at right, upper and lower florets of Bond; showing the characteristics of these varieties.

transversely in the middle portion. The Double Cross A parent was homozygous for an intermediate type of floret disjunction of the heterofracture type.

The type of spikelet disarticulation of Anthony and the absence of long bristles or basal hairs were dominant in F_1, and segregation for each pair of characters approached 3:1 in F_2. There were a few intermediate types in F_2 with fewer long hairs and smaller base than is characterized by Bond. In general, the type with Bond base and long basal hairs bred true in F_3. Progenies were obtained, also, in F_3 that bred true for the sativa type; others segregated as in F_2. Some F_3 lines were obtained that showed

unusual types of segregation in F_3. These may be illustrated by four lines, the progenies of two F_2 plants of the byzantina type of base and two of the sativa type.

F_3 line	Type of base F_2	Segregation in F_3
1	Byzantina base	28 *B*:1 *I*:1 *S*
2	Byzantina base	13 *B*:17 *S*
3	Sativa base	2 *S*:23 *I*:5 *B*
4	Sativa base	11 *S*:9 *I*:9 *B*

B = byzantina base, *I* = intermediate, *S* = sativa.

A large percentage of F_2 plants of the byzantina type of base bred true for byzantina base in F_3, and approximately one-third of those with a sativa type of base bred true for sativa base in F_3. By studying the type of breeding behavior in F_3 and later generations, it was possible to select those that were homozygous for type of base. Variations in type of segregation very probably may be correlated with variation in pairing of the chromosomes, since both sativa and byzantina oats are amphidiploids of the hexaploid type. A knowledge of genetics aids the breeder in obtaining pure breeding types and suggests the discard of those that show unusual types of segregation. After all, deviations from a 3:1 ratio are fairly common. It seems desirable to emphasize the probability that two pairs of factors are involved that are rather closely linked. Using F_2 data and placing *I* and *B* types of spikelet disarticulation together and separating for basal hairs on the basis of length of hair gave a dihybrid ratio of sativa base and short hairs, sativa base and long hairs, byzantina base and short hairs, and byzantina base and long hairs of 2118:19:81:727. By the product method, this gave a recombination percentage of 2.7 ± 0.3. It seems probable that the wide deviation from expectation of the two middle classes may be a result of abnormal chromosomal pairing or other abnormality. In the absence of long basal hairs, the type of base seemed to be a little less well developed than where the factors for byzantina base and those for long basal hairs were both present in a homozygous condition.

The sativa type of base seems more desirable than the byzantina type, because there was a definite tendency for correlation between shattering and the byzantina base.

Floret disjunction in the crosses, except where Double Cross A was one of the parents, was dependent upon at least two pairs of factors. Double Cross A, when crossed with Bond, gave segregation that was relatively well explained by a single factor pair. This factor pair was linked in inheritance with the factor pair for spikelet disarticulation and also with the factor pair for basal hairs. A knowledge of inheritance of these three pairs of factors was an aid in selecting the types that bred true. In this case, the sativa type was desired with spikelet disarticulation by fracture, short basal hairs, and sativa type of floret disjunction, the rather strong linkage aiding in obtaining pure breeding types, since more of the parental types for all three characters were obtained than would have been secured in the absence of linkage.

Quantitative Characters.—The type of results often obtained from characters that fluctuate greatly may be illustrated by plumpness of grain. When sufficient seed is available, weight per bushel is an easy character to work with. In the small grains, as studied in Minnesota, there is usually a high correlation between plumpness of grain and yield, hybrids with well-developed seeds, relatively free from shriveling, generally yielding much better, on the average, than those that show a lower degree of plumpness. Selection during the segregating generations, therefore, is made for plants with plump seeds; this is done by visual examination. Behavior in a cross between Double Cross A and Bond is used for illustration.

Parent variety or F_2	Plumpness of grain classes, per cent			
	0–25	26–50	51–75	76–100
	Number of plants in class			
Bond	1	6	54	61
Double Cross A	5	28	26	
F_2	48	102	534	381

Plumpness of grain is without doubt an inherited character, dependent upon reaction to diseases and physiological characters that may influence the metabolism of the plant. By selection during the segregation generations for freedom from disease and

for plump grain, it was possible to obtain hybrids that were resistant to all three diseases that were of the sativa type and that had plumper grain, with higher weight per bushel, than the sativa parental varieties.

Selection for yield on the individual plant basis seems of little value, since environmental conditions seem the major cause for variations. This is shown by the extreme variation in yield per plant within the parental varieties. All that can be accomplished during the segregating generations seems to be the selection for the combination of characters desired. Selection of progenies of desirable agronomic type seems a desirable practice, with the use of visual examination rather than intensive study. When homozygous lines are available, those that yield most satisfactorily can be isolated through actual comparative-yield trials.

COLCHICINE AS A POLYPLOIDIZING AGENT

Dermen (1940), in a recent review, has summarized the rather extensive literature on the methods of producing polyploids through the use of colchicine. This summary has been used freely. Heat and cold, X rays and radium, as well as ultraviolet rays, have been used by various workers to induce chromosomal aberrations. The discovery that colchicine was a satisfactory medium for inducing chromosome doubling has made its use extremely popular and has given the plant breeder a relatively efficient technic that may be used in the production of polyploid species and varieties. The technic first became available in 1937. Although there are 179 literature citations in Dermen's review, the subject of induced ploidy is of such recent origin that it is rather difficult accurately to evaluate its practical possibilities. Dermen quotes Blakeslee (1939) as follows: "We now have an opportunity to make new species to order," and " . . . the possibilities in the way of new forms of economic value seem very great." He quotes Vavilov (1939), who has said: "The possibilities opened up by the artificial induction of amphidiploidy, *i.e.*, of chromosome doubling in hybrids, are immense. Genetics is entering a new era of extensive application of distant hybridization, at least in the case of plants."

The information already available indicates that polyploids in horticultural species that can be propagated asexually may be expected to be of considerable economic value. Autopolyploids

are frequently larger in size and have more showy flowers than their diploid ancestors. Emsweller and Brierley (1940) present results with *Lilium formosanum* to show the relative ease of doubling chromosome number. They used 20 one-year-old plants, trimmed off the tip leaves when the flowering stalk was 6 or 8 in. high, and treated the apical meristem with colchicine solution for a 2-hr. period. From 31 aerial bulblets produced in



FIG. 5.—Types of snapdragons. (*A*) Tetraploid hybrid between tetraploid Velvet Beauty and tetraploid Red Shades. Note the large ruffled flowers and very deep color, also the heavier stem. The tetraploid hybrid is setting an abundance of seed, whereas the parents are highly sterile. (*B*) Triploid Velvet Beauty produced by crossing tetraploid Velvet Beauty × diploid Velvet Beauty. This plant is partially fertile. (*C*) Diploid Red Shades × Velvet Beauty. Compared with *A*, the flowers are smaller, less ruffled, and less deep in color. (*Courtesy of Nebel and Ruttle.*)

the axils of the old leaf stubs on the thickened stem apex, 22 polyploids were obtained. They state that polyploids had larger flowers, pollen grains, and stomata than diploids.

Seeds of crimson flowering tobacco, *Nicotiana sandarae*, which has nine pairs of chromosomes, were treated with colchicine, and autotetraploids were obtained by Warmke and Blakeslee (1939). The autotetraploids obtained had thicker and broader leaves than their diploid parents and grew to a larger size and produced larger

and more showy flowers. When octoploids of *Nicotiana tabacum* and *N. rustica*, which themselves are amphidiploids, were produced (Smith 1939), the resulting plants showed a general lack of vigor.

Nebel and Ruttle (1938) have pointed out the value of tetraploid marigolds, petunias, and snapdragons that were developed by the use of colchicine. In several cases, plants were obtained that were of sturdier growth and produced larger, sturdier flowers (see Fig. 5).

In the brief review given here, it is impossible to cover all phases of the economic importance of induced polyploidy. It is well known that wide crosses are sometimes possible between species and genera of grasses, although such crosses, in F_1, frequently are highly self-sterile. The development of perennial grasses of the amphidiploid type, with larger seed size, desirable for forage and range cover, through crosses between *Triticum* and *Agropyron* species is now being investigated by several workers.

In a little over a year Blakeslee and coworkers (Blakeslee 1939) succeeded in doubling the chromosome numbers of 65 different species and varieties of plants. They report doubling in the following families: Caryophyllaceae, Chenopodiaceae, Compositae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Malvaceae, Moraceae, Oxalidaceae, Plantaginaceae, Polemoniacea, Portulacaceae, Solonaceae and Violaceae.

Colchicine occurs in the corm of *Colchicum autumnale*, which may contain as much as 0.4 per cent by dry weight. A solution of 0.4 per cent in water may induce doubling in Datura, and one-thousandth of this concentration causes doubling in Portulaca. Colchicine in solution is diffusible into plant tissues, exerting its effect only on cells undergoing cell division. Colchicine prevents the formation of the mitotic spindle figure and the development of the cell wall. Cell division into sister cells is prevented, and the chromosomes continue to divide. The process of chromosome division may continue as long as the tissue is exposed to colchicine. A summary of the technics of colchicine application may be of interest.

1. Colchicine in aqueous solution diffuses through plant tissues, causing internal changes in meristematic tissues as a result of surface application.

2. Dormant tissues are not affected; only active tissues are affected by colchicine. Treatment is of value from the practical standpoint only to tissues that will develop into vegetative, sexual, or into both types of plant parts.

3. Optimum cultural conditions should be maintained during treatment so that cell division may be favored.

4. The duration of treatment must be determined for each type of material. In general, the length of treatment is dependent upon the time required to complete the cycle of cell division in the material worked with.

5. Concentration of the colchicine solution should not fall below an effective minimum and should not be sufficiently high to be fatal. A satisfactory concentration must be determined for each material.

Material that has been used with success in colchicine treatment includes seeds, seedlings, growing tips of twigs or buds or bud scales. Successful applications have been made in the following media—aqueous solution, weak alcohol, a suitable emulsion, lanolin paste, agar solution, glycerine and water, or glycerine and alcohol. The range of successful concentration that has been used varied from 0.0006 per cent to 1 per cent. Duration of treatment that has proved successful ranges from merely wetting to 24 hr.

Three methods of treatment that have proved successful will be outlined briefly.

1. Seed treatment. Seeds of *Datura*, *Cosmos*, *Portulaca*, and *Nicotiana* have been soaked in 0.2 to 1.6 per cent aqueous solution of colchicine. This treatment has been applied successfully to seeds that will germinate in a few days. Seeds may be planted after treatment and before germination.

2. Seedling treatment. Germinating seedlings may be immersed in colchicine solution in a shallow container or placed on filter paper thoroughly wetted with the solution for from 3 to 24 hr. In *Cosmos*, polyploidy was induced by moistening the soil over and around the seedlings with a 0.02 to 0.1 per cent aqueous solution after germination and before the seedlings had emerged.

3. Treating young shoots or buds. Tips of young seedlings of both woody and herbaceous plants may be treated by brushing the solution over partially exposed tips once or several times or by

immersing such material in a vessel containing the solution for the length of time necessary.

Successful treatment has been obtained by the use of 0.5 to 1.0 per cent colchicine in lanolin smeared on growing portions of young shoots and on expanding branch buds. Treatment of young seedlings of flax and petunia has been successful by brushing tepid 1 per cent colchicine agar solution (1 part 2 per cent colchicine to 1 part 3 per cent agar) over the growing tips.

CHAPTER III

MODE OF REPRODUCTION IN RELATION TO BREEDING METHODS

It is recognized that there is a close relation between mode of reproduction and methods of breeding. These facts have been emphasized by Hayes and Garber (1927). Crop plants may be placed in two groups according to mode of reproduction. These are (1) asexual and (2) sexual.

THE ASEXUAL GROUP

The most important crop plants belonging to the asexual group are potatoes, sugar cane, and many fruits. Many of the horticultural plants grown as ornamentals are members of this group also. Plants belonging to the asexual group are propagated by grafting, cuttings, layering, or other asexual means. Although this is the normal method of commercial propagation, reproduction by sexual means has occurred in asexually propagated varieties or strains of crop plants at some time in the history of their development. Vigor of growth, yielding ability, and other quantitative characters may be explained genetically, in general, as the result of the interaction of favorable, partially dominant, growth factors. With most normal quantitative characters, the number of these factors is large, and linkage is involved. These factors account for the reason that it is difficult to obtain all the desirable growth factors in any one plant in a homozygous condition. If the more promising plants are selected for propagation, it seems reasonable to expect these plants to be in a highly heterozygous condition, and the experience of breeders has shown this to be the usual case.

Clonal propagation leads usually to the perpetuation of a uniform progeny, *i.e.*, to the reproduction of the biotype, but it is recognized that gene changes or chromosomal aberrations do occur, although there is some difference of opinion regarding their frequency. Shamel, Scott, and Pomeroy (1918*a*,*b*,*c*), in Cali-

fornia, experimenting with citrus fruits, have based a system of breeding on selection and propagation from bud sports. The frequency of bud sports has been emphasized by Shamel and Pomeroy (1932), who have listed 173 cases of important bud sports in apples.

Collins and Kerns (1938) discuss mutations in the Cayenne variety of pineapple. This variety presumably originated as a vegetatively reproduced progeny of a single plant about 100 years ago. Thirty mutant types have been shown by progeny trials to reproduce themselves vegetatively; 8 types have been reproduced through sexual propagation, and 5 of these proved to be dominant characters. Collins and Kerns state, "The accumulation of mutations in asexually propagated forms may conceivably play a role in the running out and acclimatization of varieties. The parade of agricultural varieties during the past years is a demonstration of the changes going on, some of which is known to be due to progressive or regressive mutations."

Methods of breeding the asexual group may be summarized as follows:

1. Systematic survey of material.

   This is an important step in any breeding program. Such a survey includes a study of material that is already available and that can be obtained from any source whatsoever. In most breeding problems, the wild relatives deserve study also. The various steps in such a survey may be summarized as follows:

   a. Collect and grow a short row, small plot, or several individuals of the varieties of interest. Classify according to plant characters, both qualitative and quantitative.

   b. Make a systematic study of chromosome numbers and relationships.

   c. Study relationships by means of controlled crosses, using both genetic and cytologic technics.

2. Improvement by clonal selection.

   In tree fruits, a careful study of variations that appear as individual trees, or branches, is of value. A study of the transmission of these variations must be made by means of a progeny trial. All that is necessary is to compare the performance of selected variations with the normal variety. This can be accomplished rather quickly by grafting comparable trees with the selected variations and with normal budwood and making a test of the desirability of the two sources of cions. Important varieties have been selected in the past by these methods. The extent to which such selection can be made the basis of a standardized breeding program will depend upon the frequency of such mutations. The selection of budwood from healthy stock deserves consideration by all who use this method of propagation as a means of varietal increase.

In potatoes the tuber-unit or hill-selection method has been used widely. This method is of value chiefly as a means of keeping the variety free from degeneration diseases such as the various types of mosaic. It consists of studying the progeny of selected tubers or hills, selecting the most desirable clonal lines, and using these as a basis for the commercial variety. It should be recognized that bud sports do occur occasionally, and when such are observed that have selection value, they can be used as a basis for an improved variety. Blodgett and Fernow (1921) originated the tuber-index method with potatoes as a means of testing for freedom from disease. The purpose was to test by means of a tuber for the disease reaction of parent hills and eliminate the diseased hills, the test being made under greenhouse conditions during the winter months. This method is now used widely in potato-tuber selection for degeneration diseases such as mosaic.

3. Breeding plants normally propagated asexually by sexual methods.

Sexual methods of breeding plants belonging to the asexually propagated group are not widely different from those used with other crop plants. Since asexually propagated varieties are highly heterozygous, selection in self-fertilized lines is being tried as a means of obtaining parental varieties with certain desired characters in a homozygous condition. When varietal crosses are used it is of value to determine the suitability of a particular heterozygous parent variety on the basis of the characters of its progeny. Crosses between an inbred line that is relatively homozygous for certain desirable characters, with outstanding commercial varieties, often furnish the most satisfactory basis for selection of new and improved asexual varieties.

THE SEXUAL GROUP

Plants belonging to this group may be placed in several subdivisions according to their normal mode of pollination. It should be recognized that varietal differences of a genotypic nature, as well as environmental influences, are the major causes of the rather wide differences that are observed when the normal mode of pollination of plants of economic importance is studied. The following subdivisions are those of major importance: naturally self-pollinated, often cross-pollinated, naturally cross-pollinated, and dioecious.

Naturally Self-pollinated Group.

As a rule, less than 4 per cent of cross-pollination. The crops generally placed here are barley, wheat, oats, tobacco, potatoes, flax, rice, peas, beans, soybeans, cowpeas, slender wheat grass, and tomatoes.

There is a gradual variation in amount of cross-pollination from this group to that of the often cross-pollinated group and no

very clear line of demarcation between the groups. The variations that occur are a result of either environmental influences, varietal differences, or a combination of the two causes. As wide variations in the frequency of natural crosses occur from one locality to another, it seems unnecessary to summarize the many detailed studies that have been made. It is important for the breeder to learn the extent of natural crossing of the crops he is working with under his own conditions.

Methods of learning the extent of normal cross-pollination are relatively simple. With tomatoes, at the Connecticut Agricultural Experiment Station, Jones (1916) interplanted alternate plants of dwarf and standard tomatoes, at the usual spacing, in rows in the field. Seed from the dwarf plants was harvested and sown. From 2170 plants that resulted, 43, or approximately 2 per cent, proved to be of standard habit. The extent of natural cross-pollination would be therefore between 2 and 4 per cent.

Stevenson (1928) studied the extent of cross-pollination in barley under normal conditions in Minnesota, using Consul and Gatami as the parental varieties. The type characters and period of heading of these varieties are as follows:

Variety	Type character	Date heading		
		1924	1925	1926
Consul	White	6-27	6-12	6-12
Gatami	Black	6-26	6-12	6-15

Seed of the two varieties was sown alternately in rows spaced 1 ft. apart. Black is dominant over white, and the extent of natural crossing was determined by sowing seed of the white glumed variety, collected under the conditions described, and determining the number of natural crosses. Results from the 3 years are as follows:

Year	White glumed plants	Black glumed plants	Per cent off type
1924	2878	1	0.04
1925	1600	2	0.12
1926	2012	3	0.15

From similar studies, no natural crosses occurred between Hanna and Jet, Oderbrucker and Lion, and Manchuria and Nepal.

Natural crossing has been studied extensively in wheat. There is a rather wide range in the amount of natural crossing as reported by investigators located in various parts of the world where wheat improvement has been carried on. Early investigators, including DeVries, Biffin, and Fruwirth, considered that natural crossing was very infrequent. Nilsson-Ehle, in Sweden, stated that some varieties are cross-pollinated much more frequently than others. Natural crossing at University Farm, St. Paul, Minnesota, of at least 2 to 3 per cent, on the average, has been observed. Powers (1932) studied natural crossing in Marquillo spring wheat, derived from a cross of Iumillo *durum* with Marquis. Marquillo was grown in alternate rows with Ceres, and the percentage of natural crosses determined by inoculation in the seedling stage with physiologic race 21 of black-stem rust, *Puccinia graminis tritici*, to which Marquillo normally is resistant and Ceres susceptible. Seedlings from seed produced on non-covered spikes of Marquillo showed 3.6 ± 0.50 per cent of susceptible plants. Since susceptibility is dominant over resistance to form 21 in crosses of Ceres $\times$ Marquillo, it is fair to conclude that natural crossing to the extent of 7.2 per cent occurred in Marquillo wheat during the year that the study was conducted.

2. Often Cross-pollinated Group.

> In this group, self-pollination is more frequent, as a rule, than cross-pollination, although cross-pollination may occur so frequently that some method of preventing cross-pollination between varieties and strains of different genotypic constitution must be followed throughout the breeding and seed-distribution program. Crops belonging to this group are cotton, sorghums, and some strains of sweet clover.

Except for the necessity of controlling pollination in seed plots to a greater extent than with the self-pollinated group, it seems probable that methods of breeding are not greatly different than for the self-pollinated group.

Before starting a hybridization program of improvement, it may be desirable to practice self-pollination and selection in order

to isolate the more desirable homozygous types as parents and eliminate the less desirable variations.

3. Naturally Cross-pollinated Group.

Important crop plants placed in this group include maize, rye, clovers, sunflowers, sugar beets, many fruits, some annual and most perennial grasses, cucurbits, Brassica species, most root vegetables.

This group is composed of plants of widely different habit in relation to mode of pollination. It includes such plants as maize, with which cross-fertilization is the rule and which sets seed freely when artificial self-pollination is practiced. The wind-pollination habit and the large amount of pollen produced tend to cause cross-pollination that approaches 100 per cent. Then there are many plants adapted to insect pollination, in which cross-pollination, under normal conditions, is essential to seed production, and many plants that are partially or wholly self-incompatible, in which case cross-pollination is essential to seed production because of self-sterility.

Self-sterility and other causes of self-unfruitfulness will be discussed in much greater detail in connection with the presentation of methods of breeding crop plants that normally do not set seed by self-pollination.

It is apparent that many crop plants contain genotypes that carry factors both for self-fertility and sterility. Where self-sterility is the rule, methods of breeding are not widely different than in dioecious plants, since two parent plants must be selected in order to obtain a progeny.

4. Dioecious Plants.

Important crop plants of this group are hops, hemp, date palm, spinach, and asparagus.

In breeding plants belonging to this group, it is necessary to select both male and female plants with the characters desired and test their progeny to determine the breeding value of particular parents. By this means varieties of superior type may be synthesized.

SELF-POLLINATION LEADS TO HOMOZYGOSIS

Even though only an occasional natural cross occurs in a normally self-pollinated crop, this may lead to a new combination

of characters and thus be a source of material for selection. It will be of interest to show what will happen in later generations of self-fertilization as a result of a cross between varieties differing by one or more genetic factor pairs. Two somewhat different formulas have been used to express the expectations (East & Jones 1919).

Suppose that the two parent varieties differ by several factor pairs. The following formula, $[1 + (2^r - 1)]^n$, may be used where r equals the number of segregating generations after a cross and n equals the number of independently inherited factor pairs involved and the first and second terms of the binomial are 1 and $2^r - 1$, respectively. The exponent of the first term gives the number of heterozygous factor pairs and the exponent of the second term, the number of homozygous factor pairs. Supposing the number of factor pairs is 3, *i.e.*, $n = 3$, and the progeny is in the fifth segregating generation, or F_6, *i.e.*, $r = 5$ and $2^r - 1 = 31$. The results will be $1^3 + 3(1)^2 31 + 3(1)(31)^2 + 31^3$, giving:

1 individual with all three factor pairs heterozygous.

93 individuals with two factor pairs heterozygous and one homozygous.

2883 individuals with one factor pair heterozygous and two homozygous.

29,791 individuals with all three factor pairs homozygous.

Another formula that has been used to express the percentage of homozygous individuals in any generation following a cross between different forms is $\left(\frac{2^r - 1}{2^r}\right)^n$, where n and r have the same meaning as in the previous formula. In actual practice, the calculated expectation would not hold unless all the progeny of each genotype were equally productive and the factor pairs were independently inherited. If linkage is involved, this changes the percentage of homozygous individuals but does not change the percentage of homozygosis, as has been shown by Wright (1921). The percentage of homozygosis in any segregating generation, r, can be obtained by the foregoing formula for a single factor pair. Under conditions of self-pollination, linkage increases the rapidity of obtaining homozygous individuals over that expected for independent Mendelian inheritance.

The results of applying this formula with 1, 5, 10, and 15 factor pairs for from 1 to 10 generations of self-fertilization have been

expressed in the form of curves by Jones (1918). The results (Fig. 6) are given on the basis of the percentage of heterozygous individuals in each selfed generation and the percentage of heterozygous pairs, *i.e.*, the percentage of heterozygosis.

These graphs show that self-fertilization leads rapidly to homozygosis and that the progeny of individual plants of a self-fertilized crop may be expected to breed true for the most part.

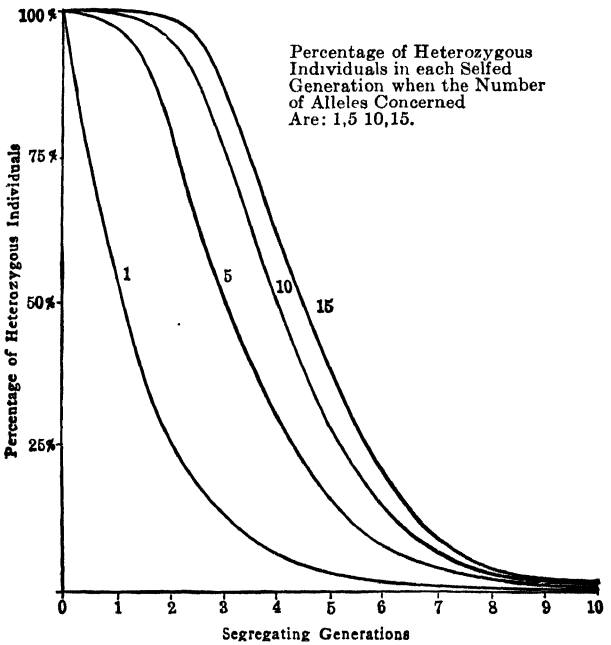


FIG. 6.—The percentage of heterozygous individuals in each selfed generation when the number of independently inherited factor pairs are 1, 5, 10, and 15. The percentage of heterozygosis in any selfed generation is given by the curve for one factor pair.

The principles outlined show why breeding methods have been standardized to a considerable extent with self-pollinated crop plants.

THE EFFECTS OF SELF POLLINATION IN THE OFTEN CROSS-POLLINATED GROUP

It has been stated that except for the necessity of greater care in controlling pollination the breeding of the often cross-pollinated group can be carried on in much the same manner as with plants belonging to the naturally self-pollinated group.

Kearney (1923), with Pima cotton, studied the effects of controlled self-fertilization during successive generations. Some of the results presented by him are summarized here.

TABLE 2.—RANDOM SAMPLE OF COMMERCIAL STOCK OF PIMA COTTON COMPARED WITH STOCK INBRED FOR SEVEN SUCCESSIVE GENERATIONS

Population	Flowers tagged	Percentage of bolls shed	Mean number of seeds matured per boll	Mean weight of 1000 seed, g.	Percentage of germination of seeds
Inbred.....	296	11.8 ± 1.3	17.2 ± 0.12	13.6 ± 0.04	90.8 ± 0.8
Open pollinated	367	8.4 ± 1.0	17.1 ± 0.12	13.4 ± 0.03	90.2 ± 0.9
Difference ..	...	3 4 ± 1.6	0.1 ± 0 17	0.2 ± 0.05	0.6 ± 1.2

Boll Weight and Lint Index

Population	Number of bolls	Seed cotton	Lint index
Inbred.....	105	3.22 ± 0.21	4.90 ± 0.27
Open pollinated	115	3.04 ± 0.06	5.12 ± 0.03
Difference.....	...	0.18 ± 0.22	0.22 ± 0.27

Boll Dimensions

Population	Number of bolls	Length, mm.	Diameter, mm.
Inbred.....	25	46.6 ± 0.56	26.8 ± 0.19
Open pollinated.....	25	45.7 ± 0.80	26.1 ± 0.19
Difference.....	...	0.9 ± 0 97	0.7 ± 0.27

There was no harmful effect of continued self-pollination in this variety of cotton. It may be concluded that controlled self-pollination can be used, when desired, with plants belonging to this group without leading to a great reduction in vigor.

Humphrey (1940) has emphasized the desirability of inbreeding cotton in order to obtain uniformity in fiber characters. Comparison of lines that had been self-pollinated for 2 and 7 years indicated that inbred lines were much more uniform than the variety from which they arose, but little increase in uniformity was obtained after 2 years of self-pollination. Humphrey's data lead to the conclusion that vigorous self-pollinated lines can be

obtained in cotton, and, as would be expected, there seem to be no harmful effects of continued self-pollination.

EFFECTS OF SELF-FERTILIZATION IN CROSS-POLLINATED PLANTS

From the genetic standpoint, artificial self-pollination in a normally cross-pollinated crop leads to the production of homozygous lines. In many crops, notably corn, there is a rapid reduction in vigor when self-pollination is practiced. The extent that vigor of growth is reduced is not the same in all lines, and some inbred lines of corn have been obtained that appear relatively homozygous and are rather vigorous. In general, in corn, no inbred lines that approach homozygosis have been obtained that are as vigorous as normal corn. Studies of the effects of self-fertilization have been made with many crop plants. Extensive studies of squashes have been made, and much of the improvement in recent years has resulted from the isolation of desirable selfed lines and their use as commercial varieties. Both high-yielding and low-yielding selfed lines have been isolated. Cummings and Jenkins (1928) studied a high-yielding line that had been selfed for 10 generations without harmful effect.

It is apparent that the extent to which a crop can be inbred without leading to a great reduction in vigor will be the main factor in deciding how extensively controlled self-pollination can

TABLE 3.—THE EFFECT OF 30 GENERATIONS OF SELF-FERTILIZATION WITH THREE INBRED LINES OF MAIZE UPON THE HEIGHT OF PLANT AND YIELD OF GRAIN

Number of generations selfed	Line 1-6		Line 1-7		Line 1-9	
	Height, in.	Yield, bu. per acre	Height, in.	Yield, bu. per acre	Height, in.	Yield, bu. per acre
0	117	81 ± 7	117	81 ± 7	117	81 ± 7
1–5	87	64 ± 11	81	51 ± 7	77	41 ± 5
6–10	97 ± 1*	45 ± 12	84 ± 1	36 ± 5	82 ± 2	34 ± 4
11–15	97 ± 3	38 ± 4	84 ± 2	34 ± 3	83 ± 2	26 ± 2
16–20	88 ± 4	22 ± 4	85 ± 3	24 ± 3	75 ± 4	14 ± 3
21–25	81 ± 2	20 ± 6	75 ± 3	21 ± 3	71 ± 3	13 ± 2
26–30	92 ± 3	24 ± 9	80 ± 2	18 ± 4	77 ± 3	9 ± 4

* Standard errors.

be used in breeding cross-pollinated plants. Studies of controlled cross- and self-pollination with each of the important crop plants are essential in the establishment of breeding methods.

Jones (1939) has summarized the effects of continued inbreeding with maize for three inbred lines started by East in 1905 and discussed by East and Hayes (1912). Yield in bushels per acre and height of plant in inches given in Table 3 were presented by Jones (1939). The data were given as averages for 5-year periods to overcome seasonal fluctuations.

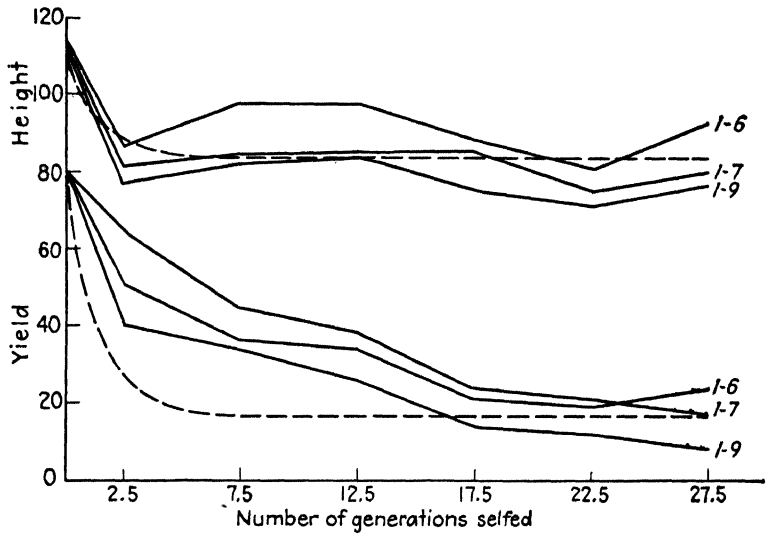


FIG. 7.—A comparison of three maize lines, derived from the same variety, self-fertilized for 30 generations. Height of stalk is measured in inches and yield of grain in bushels per acre, both plotted on the same scale. The broken lines are the theoretical curves of inbreeding. (*Adapted from Jones.*)

The results are presented also in the form of curves in Fig. 7.

The theoretical curves were calculated by subtracting the average height and average yield of the three inbred lines at the end of the 30 generations of self-pollination from the figures at the start. The difference was halved in each generation and subtracted from the initial yield. Theoretical yields at the end of the fifth generation of inbreeding were obtained by averaging the theoretical yields at the end of each generation from 1 to 5, with the use of the following calculations, where the original yield was 81 bu. and the average yield at the end of 30 years of selfing was an average of 24, 18, and 9, or 17 bu. Subtracting from 81,

one obtains 81 − 17 = 64. This value is halved for each successive generation of selfing and subtracted from 81. On this basis, theoretical yields for each of the first five generations of selfing can be computed as follows:

Generation of selfing	Calculation	Theoretical yield
1	81 − ($\frac{1}{2}$ × 64)	49
2	81 − ($\frac{3}{4}$ × 64)	33
3	81 − ($\frac{7}{8}$ × 64)	25
4	81 − ($\frac{15}{16}$ × 64)	21
5	81 − ($\frac{31}{32}$ × 64)	19
	Average...	29.4

The calculations are based on the hypothesis that the effects of inbreeding are dependent upon the extent of heterozygosity, the number of heterozygous pairs of factors being reduced one-half for each successive generation of self-fertilization. The average theoretical yield of the three inbred lines, for generations 1 to 5, of 29.4 bu., was less than the actual yield obtained, which indicates that selection was practiced.

Comparing the actual and theoretical curves indicates that the three inbred lines were homozygous for factors influencing height after 5 generation of selfing and for yield after approximately 20 generations of selfing. Besides these lines that can be propagated by continued self-pollination, there are other inbred strains that are so weak that they cannot be propagated and still others that can be perpetuated only with difficulty.

HETEROSIS AND ITS EXPLANATION

Early plant hybridists, including Kölreuter, in the eighteenth century, Gärtner and Weigmann, in the nineteenth, noted the increased vigor of hybrids. Although many others in the last century observed hybrid vigor, a clear understanding of the effects of self-fertilization in cross-pollinated plants and of the effects of crossing self-pollinated plants was obtained only as a result of genetic research. East and Hayes (1912) pointed out the value of hybrid vigor both in evolution and in plant breeding and Shull (1914) suggested the term heterosis in the following words:

To avoid the implication that all the genotypic differences which stimulate cell division, growth and other physiological causes are Mendelian in their inheritance and also to gain brevity of expression, I suggest that instead of the phrases, "stimulus of heterozygosis," "heterozygotic stimulation," . . . , that the word heterosis be adopted.

Because of the importance to the plant breeder of heterosis, it seems desirable to summarize the suggestions for an explanation of heterosis in the light of present-day knowledge.

Keeble and Pellew (1910) explained hybrid vigor in peas from a cross between two half-dwarf varieties, Autocrat and Bountiful, on a dihybrid basis with dominance in the F_1 of the thick stem of one parent and long internode of the other.

This explanation was not considered generally applicable by East and Shull, since other cases of hybrid vigor could not be placed on an equally simple basis. At this time (around 1910) there was a lack of appreciation by most workers that for many characters large numbers of factor pairs were involved. Brief quotations from East and Hayes (1912) serve to summarize the viewpoints of East and Shull, who, in general, were in close agreement.

> One can say that greater development stimulus is evolved when the mate of an allelomorphic pair is lacking than when both are present in the zygote. In other words, development stimulus is less when like genes are received from both parents.
>
> The decrease in vigor due to inbreeding naturally cross-fertilized species and the increase in vigor due to crossing of naturally self-fertilized species are manifestations of the same phenomenon. This phenomenon is heterozygosis. Crossing produces heterozygosis in all characters by which the parent plants differ. Inbreeding tends to produce homozygosis automatically.
>
> Inbreeding is not injurious in itself but weak types kept in existence in a cross-fertilized species through heterozygosis may be isolated by this means. Weak types appear in self-fertilized species, but are eliminated because they must stand or fall on their own merits.

The selfed lines of corn first grown by East at the Connecticut station in 1906 and carried on by Hayes from 1910 to 1914 were a part of the long-time selfed material used by Jones (1918) in studies of selfing and crossing.

Studies of size inheritance in tobacco and corn furnished a considerable part of the necessary evidence that many quantita-

tive characters were dependent upon the interaction of many factors for their full expression. If such is the case, it is evident from any particular cross that it is difficult to obtain all factors in a homozygous condition that have an influence on hybrid vigor. The explanation of heterosis given by Jones (1917) is well known to all students of genetics. He supposed that the vigor of an F_1 cross was dependent upon the interaction of dominant, favorable growth factors, part of which were obtained from each of the two parents. If large numbers of factors are involved, linkage is bound to occur, and this makes it extremely difficult to obtain all necessary growth factors in a homozygous dominant condition in later segregating generations.

To the breeder who has had much experience with quantitative inheritance the explanation seems entirely logical. It is difficult to prove the truth or falsity of the explanation. To the breeder of economic crop plants the dominance of linked growth factors in relation to heterosis furnishes a basis for methods of breeding cross-pollinated plants and the perpetuation of hybrid vigor to the extent possible with each particular category of crop plant.

Collins (1921) raised certain objections to Jones's explanation. He emphasized the importance of deleterious recessives that so frequently show up as a result of inbreeding maize. He pointed out also that the effect of a genetic factor was dependent upon the size of an organism and that skewness of the F_2 distributions would not be evident if as many as 20 pairs of factors were involved, with complete dominance of each and a cumulative effect of one on the other. With more pairs of factors involved, linkage would result, however, and add to the difficulty.

Richey (1927) and Richey and Sprague (1931) have presented data on convergent improvement that gives some support to Jones's explanation of hybrid vigor. The method of convergent improvement will be presented in much greater detail in relation to corn breeding. It is equivalent to double backcrossing and furnishes a method for improving each of two inbred lines without interfering with their combining ability. If the selfed lines A and B combine to give a vigorous F_1 cross, $(A \times B)$, two series of backcrosses are carried on, $(A \times B)A$ and $(A \times B)B$. In the cross of $(A \times B)A$ and subsequent backcrosses to A, it is hoped to retain the favorable dominant growth factors from A and add a part of B. In the cross of $(A \times B)B$, etc., the favorable domi-

nant growth factors of B will be retained, and a part of these from A will be added. After backcrossing, selfing is necessary until the heterozygous dominant growth factors become homozygous. The F_1 cross of $A(B_1)$, containing the growth factors of A with a part of those obtained from B, with $B(A_1)$, or $[A(B_1) \times B(A_1)]$, should yield as much as $A \times B$ if the partially dominant linked-growth-factor theory of hybrid vigor is the correct one. Greater yields seem possible if dominance is not complete.

Richey and Sprague (1931) presented data from six crosses where N = nonrecurring parent, R = recurring parent, and $(N \times R_4)$ refers to four generations of backcrossing of $(N \times R) \times R$. All combinations of new crosses $[A(B_1) \times B(A_1)]$ should not be expected to yield equally as well as $A \times B$, and in practice it is necessary to determine the number of generations to backcross before selfing. One of the crosses studied by Richey and Sprague indicates the possibility of increasing the yield of selfed lines and of the F_1 cross over the original selfed lines and F_1 cross, respectively, by the process of convergent improvement. In a replicated trial, $N \times R$ yielded 17.8 $\pm$ 0.20 lb. of ear corn per plot, $N \times (N \times R_4)$, an F_1 cross of a fourth generation backcross of $(N \times R)R$, when crossed with N, yielded 19.0 $\pm$ 0.30, or significantly more than $N \times R$. The yield of R selfed was 5.5 $\pm$ 0.22, which is significantly lower than the yield of $(N \times R_4)$ of 8.3 $\pm$ 0.24.

The writers say, "Convergent improvement, suggested originally from theoretical considerations as a means of improving selfed lines of corn without interfering with their behavior in hybrid combination, so far has been found successful. Furthermore, the results suggest that this method may also provide a means by which the yields of F_1 crosses between selfed lines can be raised to an even higher level."

More recently East (1936*b*) has presented a genetic explanation for heterosis that emphasizes the importance of linkage and makes the suggestion that multiple alleles are concerned also in heterosis. The genetic factors involved are not those used normally in genetic experiments, called physiologic defectives by East, but the factors with small effects and more difficult to study are considered to be of greater importance in evolution and in plant breeding. It is suggested by East that these factors, which have a cumulative effect and for which dominance is

virtually absent, occur in series of multiple alleles. Each member of a series may be considered to have the ability of affecting a different physiological process. Thus, if A_1, A_2, and A_3 are three such alleles, A_1A_2 or any other combination of two of the three factors would have a greater effect than the homozygous condition for one, *i.e.*, A_1A_1, A_2A_2, or A_3A_3. Although there is nothing inconsistent in such a hypothesis in the light of the numerous series of multiple alleles for qualitative characters, there seems no reason to suppose that multiple alleles are of greater importance for quantitative than for qualitative characters.

Studies of corn breeding have given further evidence regarding hybrid vigor, although the problem of heterosis needs further study, both on a genetic and physiological basis. It is now generally accepted by students of corn breeding that combining ability is a genetic character. Recent rather extensive studies of Hayes and Johnson (1939) showed the extent to which combining ability is an inherited character. When selfed lines were selected from a cross between inbreds with high combining ability, most of the selfed lines obtained from the cross were of high combining ability also, as tested in inbred-variety crosses. Conversely, when selfed lines were selected from a cross of low combiners, the greater proportion of lines obtained were of low combining ability. Data were given to show that the characters of selfed lines that measure vigor of growth were responsible for approximately 45 per cent of the variance in yield of the inbred-variety crosses.

Heterosis, then, is a general term for hybrid vigor. It is a phase of quantitative inheritance, and if quantitative inheritance is Mendelian it seems equally reasonable to place heterosis in a similar category. If the growth characters of self-pollinated plants are inherited in the same manner as in cross-pollinated plants, it seems evident that nature and man have obtained vigorous self-pollinated plants by selection of the fittest. It is evident that similar selection in selfed lines of cross-pollinated plants will, in many cases, lead to the isolation of inbred lines that are progressively more vigorous than those now available. The extent of improvement obtainable can be determined only by actual study.

In recent years, physiological studies of the manifestations of heterosis have been made with several different crops by Ashby

(1930, 1932, 1937), Sprague (1936), Lindstrom (1935), Luckwill (1937), *et al.* In general, three stages of development may be differentiated: (1) fertilization to maturity of seed; (2) from germination to first flowering; (3) subsequent growth. The efficiency of the F_1 crosses was studied for various physiological characters. The hybrids during the stages of development designated as (2) and (3) did not excel the better inbred parent in relative growth rate, respiration rate, or assimilation rate. Ashby attributes the greater development of the hybrid to "greater initial capital," *i.e.*, greater embryo size. Although Ashby's data were in agreement with this hypothesis, no such relation is universally present. Sprague (1936) concluded that growth rate of the hybrids was greater than that of the inbreds during the first stage and in the early seedling stage but could not demonstrate a higher growth rate for the hybrids from the late seedling stage to maturity.

Kiesselbach (1922) studied external and internal expressions of hybrid vigor in maize crosses. The increased weight of kernel due to crossing showed the following percentage of increase of parts of the hybrid kernel over the kernels of the inbreds: total kernel, 11.1 per cent; embryo, 20.2 per cent; endosperm, 10.4 per cent; and seed coat, 5.4 per cent.

Some measurements of the causes of increased vigor given by Kiesselbach are of interest.

Increase of hybrids over their pure-line parents:

Stalk diameter at base, 48 per cent.

Number of fibro-vascular bundles in cross section of stalk, 43 per cent.

Number of fibro-vascular bundles in 1 sq. cm. of cross section, −38 per cent.

Average diameter of one pith cell in stalk, 6 per cent.

Average length of one pith cell in stalk, 10 per cent.

Number of pith cells along one diameter in cross section, 38 per cent.

Increase in size of the hybrid over the parents in pith cells in the stalk and epidermal cells of the leaf was studied in relation to cell number and cell size. The total increase of the hybrid over its parents was due to 10.6 per cent increase in cell size and 89.4 per cent to an increase in cell number.

Bindloss (1938), Whaley (1939*a*,*b*), and Wang (1939) studied the apical meristem of inbreds and F_1 hybrids without finding any one characteristic uniformly correlated with hybrid vigor. Bindloss observed a positive correlation between nuclear size and heterosis in one maize pedigree but no such relation in two others studied. Her data indicate significantly larger nuclei for the hybrid than for either parent in one cross, but in another hybrid the nuclei in the meristem were intermediate between the two inbred parents. Whaley found that cell and nuclear size in the plumular meristem of *Lycopersicum* decreases during development but less rapidly in the hybrids than in their parents. The differences observed indicate a fundamental metabolic difference between the hybrids and their parents. Wang studied four inbred lines of corn and all six possible F_1 crosses between them, using the apical meristem of the growing shoot. He found some evidence of heterosis in the volume of the plumular meristem and within the hybrids or within the selfed lines a positive correlation between cytonuclear ratio of the cells of the growing shoot and vigor of growth. This ratio, however, did not hold when comparisons of hybrids and selfed lines were made.

From these physiological studies, there is an indication that the hybrid approaches the better parent in measures of physiologic efficiency. The lack of agreement among the various studies indicates that heterosis is manifested in various ways in different hybrids and that it may be due to various causes. The hypothesis of the complementary action of growth genes seems the best genetic explanation now available. For the plant breeder, the explanation of Jones for heterosis on the basis of the partial dominance of linked growth factors furnishes, at any rate, a working basis that aids in an attack on improvement problems. Considering heterosis as a phase of quantitative inheritance furnishes a basis for an outline of methods of breeding that aim to obtain, as far as possible, the full benefits of hybrid vigor to the grower and producer of crop plants.

A CLASSIFICATION OF METHODS OF BREEDING SEXUALLY PROPAGATED PLANTS

A brief outline of methods of breeding will help to illustrate the close relation between methods of breeding and mode of pollination. The major groups are as follows:

I. Introductions.
II. Selections.
  A. Mass selection.
    1. In self-pollinated crops.
    2. In cross-pollinated crops.
    3. In dioecious crops. Selection of both male and female plants for the characters desired.
  B. Individual plant selection.
    1. In self-pollinated crops.
    2. In cross-pollinated crops without control of pollination.
    3. In controlled self-pollinated lines of cross-pollinated plants.
    4. In dioecious crops.
    5. In crops normally clonally propagated.
III. Hybridization.
  A. Crosses in self-pollinated crops.
    1. The pedigree and bulk methods.
    2. Backcrossing.
  B. Crosses of self-pollinated lines and the use of the F_1 generation for the commercial crop.
  C. Convergent improvement.

These various methods will be outlined in greater detail later. At this time it will be sufficient to discuss them briefly.

Introduction is not a method of breeding in itself but a means of securing material from other workers and from foreign countries. Many species and varieties of crop plants now grown in one country were introduced originally from foreign countries. For example, the soybean, introduced into the United States from the Orient in the present century, is becoming of outstanding value to American agriculture.

Mass selection as now practiced in self-pollinated crops is chiefly a matter of roguing or of selecting individual plants or heads from a commercial standard variety for seed-plot purposes. In cross-pollinated plants, mass selection is of great value as a means of selecting and developing ecotypes that through years of natural selection have become adapted to particular environmental conditions. Grimm alfalfa, selected in Carver County, Minnesota, many years ago, was a product of mass selection.

More varieties of self-pollinated crops have been obtained from the individual-plant method of selection than by other methods. Some of the results of these and other methods of breeding have been summarized by Hunter and Leake (1933). Most commercial varieties are mixtures of different biotypes that

can be isolated by the individual-plant-selection method. These mixtures result from natural crossing, mutation, or from mechanical mixtures. They have furnished a logical basis for the selection of pure-line strains of greatest promise. Several varieties of oats, Gold Rain and Victory at Svalöf, Sweden, Gopher in Minnesota, Richland, Iowar, and Iogold from Iowa, and Rusota from North Dakota aré illustrations of valuable varieties obtained by this method of breeding.

With cross-pollinated crops one of the best known illustrations of individual-plant methods of selection is the ear-to-row-selection method with corn outlined by Hopkins about 1900. This method has been used rather widely as a means of developing adapted varieties of corn. Most of the improvement in sugar content and quality, of a heritable nature, with sugar beets was a result of individual-plant selection without control of pollination.

With cross-pollinated plants like corn, selection in self-fertilized lines has been used during the last 15-year period as one of the steps in the modern corn-breeding program. It has been used also with potatoes as a means of developing better breeding stock.

With dioecious plants, a good illustration of the individual-plant methods of selection that led to the development of an improved variety is the Washington asparagus listed in many seed catalogues. In this case, both male and female parent plants were selected and their combining ability determined.

Hybridization is a means of combining the desirable characters of two or more varieties. Two smooth-awn varieties of barley—Velvet, developed at the Minnesota Agricultural Experiment Station, and Barbless, in Wisconsin—and the Little Joss and Yeoman varieties of wheat developed in England are illustrations of the many cases in recent years where new varieties of crop plants have been developed by combining in a single variety the desirable characters of two or more parents.

The development of a hybrid method of seed-corn production was predicted by Shull in 1909. Some of the results of this method of breeding have been emphasized in the first chapter.

As a result of combined genetic and plant-breeding studies, the value of backcrossing in plant breeding is becoming generally recognized. When it is desired to add one or two characters to

an otherwise desirable variety and the technic of crossing is relatively easy, the method seems almost to be made to order.

Convergent improvement or double backcrossing is a method of improving each of two inbred lines of corn or other crop plant without modifying their combining ability.

These and other methods of plant breeding will be discussed in greater detail in later chapters, when the relative desirability of various methods of breeding for different types of improvement problems will be emphasized.

CHAPTER IV

TECHNICS IN SELFING AND CROSSING

Two general methods for the exclusion of foreign pollen may be used in controlled self-pollination. One method is the use of space isolation, spacing single plants far enough apart from other plants with which they might cross so that selfing is ensured. The distance needed for complete isolation will vary with the crop, weather conditions, and natural barriers to the spread of pollen. This method has been employed rather extensively in selfing sugar beets. The other method is the use of some type of bag, either paper, vegetable parchment, or cloth, to enclose the inflorescences and ensure self-pollination.

Crossing different strains usually involves the use of some special technic appropriate for the crop and environmental conditions prevailing. A knowledge of flower structure of the species or variety to be worked with is essential before crossing is undertaken. Some important features of the technic of crossing have been summarized by Hayes and Garber (1927) as follows:

1. Make a careful study of the structure of the flower before commencing operations. This may be with, or without, the aid of a dissecting microscope.

2. Determine which flowers produce the larger, healthier seeds and which set seed most freely.

3. Learn the normal time and method of blooming of the flowers and the length of time that the pistil will remain receptive and the pollen grains capable of functioning.

4. Procure the necessary instruments, and see that these are of an efficient kind for the work to be undertaken.

5. Be careful not to injure the flowering parts any more than is necessary. Do not remove the surrounding flower parts, *i.e.*, petals, glumes, etc., unless necessary.

6. A few crosses carefully made are of much greater value than many pollinations carelessly executed.

Some of the common methods employed in selfing and crossing different crops will be outlined.

Corn.—In selfing and crossing individual plants of corn, vegetable parchment and paper bags are commonly used to cover the ears and tassels. At Minnesota, ear bags made of 40-lb. vegetable parchment paper, 4 by 2½ by 11 in. in size, with round bottom, 1-in. lip, and 1-in. bottom fold, sealed with a double strip of casein glue, have proved very satisfactory. These bags are placed over the ears before the silks emerge and are clipped with a collette paper clip to the stalk. After emergence of the silks, another bag, made of extra-heavy kraft paper, 7 by 4¾ by 16 in., with round bottom and 1-in. lip, is placed over the tassel. The end of the bag is folded tightly around the stalk and held in place with a paper clip. The next day the ear bag is removed, and the pollen that has collected in the tassel bag is poured over the silks of the ear to be selfed or crossed. In some cases, it is desirable to clip off the young silks at about the time that the tassel bag is placed over the tassel. This ensures a tuft of silks of similar length at pollination time. After pollination, the parchment ear bag is replaced and tied to the stalk with a string. This bag is left on until harvest. The used tassel bag is discarded.

A method used commonly is to cover the ear shoot with a glassine bag, approximately 6½ by 2½ in., before the silks appear. These bags are placed over the ear shoot but are not clipped or tied to the plant. This method works satisfactorily when the ear shoot is large enough to support the bag. In some early varieties and inbreds of field corn and some strains of sweet corn and popcorn, the ear shoot is not sufficiently well developed to hold a glassine bag in place. After the silks appear, a specially treated tassel bag that is resistant to moisture and weathering is placed over the tassel and held in place with a clip. At pollination, the bottom of the glassine bag is clipped off, the silks are pollinated, and the ear shoot, after pollination, is covered with the tassel bag that is clipped in place.

Another method used in selfing is the "bottle method" developed by Jenkins (1936). Small glassine bags are placed over the ear before any silks appear. After emergence of the silks has begun, a 2 oz. bottle of water is hung on the stalk at the ear-bearing node with a bent wire. The tassel is cut from the stalk, its shank is inserted in the bottle of water, and tassel

and shoot are enclosed in a large paper bag. The tassel should

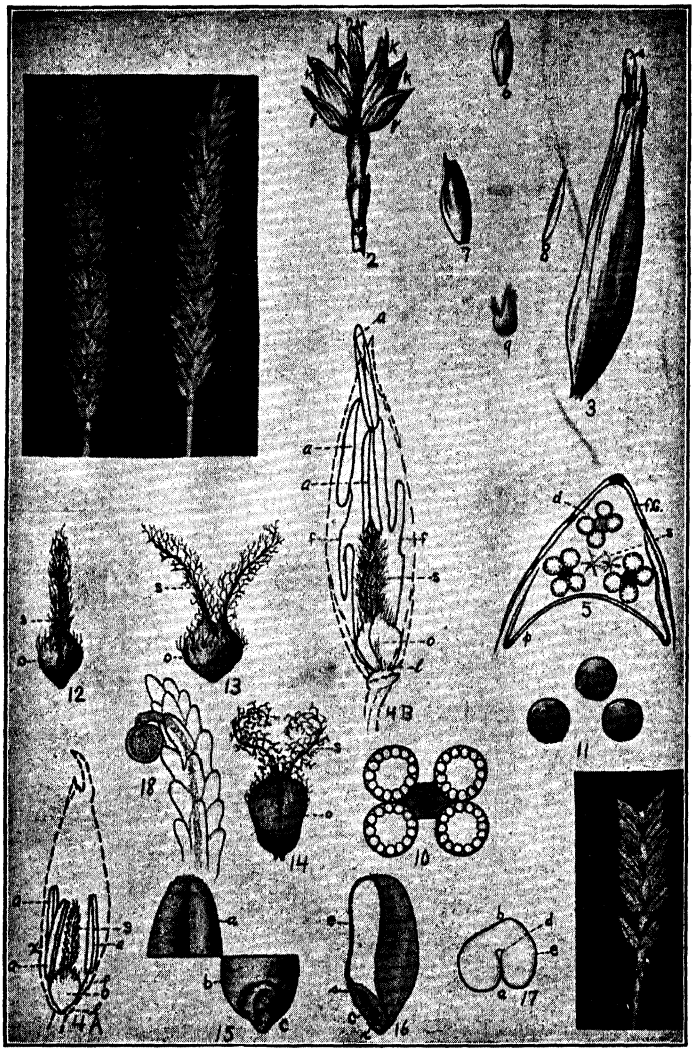


FIG. 8.—Details of wheat inflorescence.

Upper left, normal spikes; lower right, emasculated spike; 2, spikelet natural size; *f* and *g*, flowerless glumes; *k* and *r*, florets; 3, a single flower closed just after flowering, 3×; 4*A*, longitudinal diagram before flowering, x 2.5×, *a* = anthers, *o* = ovary, *s* = stigma, *f* = filament; 4*B* = diagram after flowering; 5 = transverse floral diagram, 6×, *fg* = lemma, *p* = palea, *a* = anthers, *s* = stigma; 6, flowerless glume, 7, lemma, 8, palea, slightly reduced; 9, lodicule, 4×; 10, cross-section anther, 26×; 11, pollen grains; 12, ovary and stigma just prior to flowering; 13, at flowering; and 14, shortly after; 15, 16, 17, the mature seed. (*After Babcock and Clausen*, 1918, *after Hays and Boss.*)

be arranged directly above the ear shoot. The bottle of water seems to keep the tassel alive and shedding pollen as new silks

emerge. After 48 to 72 hr., the tassels may be removed and the bottles collected.

When large quantities of seed are required, as in sib pollinations or crossing, it is usual to mix the pollen collected from several plants of one line and apply the mixture to the silks of the desired number of plants in the female line. For this purpose, a small "pollen gun" or small insect duster may be used to apply the pollen, the anthers first being screened out.

Large-scale production of crossed seed is accomplished by planting the lines to be crossed in alternate blocks in a field isolated from other corn and removing the tassels of the female line before pollen sheds or before the silks appear. The seed produced on the detasseled line is hybrid seed. The ratio of pollen-parent rows to detasseled rows varies from 1:2 to 1:4, depending on the pollen-producing ability of the male line.

Wheat, Oats, and Barley.—Hayes and Garber reviewed studies of blooming with wheat that emphasize the importance of this knowledge in relation to time of emasculation. The period from about 5 p.m. to 7 a.m. was referred to as night. Of 2,977 flowers studied on 69 spikes, 1,492 bloomed at night and 1,485 during the day. These data show that with wheat it is equally satisfactory to pollinate during the day as in the very early morning.

The same writers reviewed studies that have been made to determine whether it was necessary to cover emasculated spikes of wheat. All results showed that emasculated spikes when left uncovered without hand pollination set a high proportion of seed.

Crosses in the self-pollinated cereal grains may be made either in the field or greenhouse. All but about 8 to 15 of the florets on a spike or panicle are removed, before anthesis, from the heads of each plant to be used as a female parent. The stamens are then removed from these remaining flowers with small forceps before the anthers dehisce, and the head is enclosed in a paper bag. Bags 2½ in. wide and 6 in. long, made of vegetable parchment paper, are satisfactory. About 2 days later, ripe anthers are collected from the male plants, and pollen is applied to the flowers of the female by breaking a mature anther and placing it within the emasculated floret. The paper bag is placed over the pollinated head and allowed to remain until harvest. Seed from both male and female

plants used in the crosses may be harvested also. Direct comparisons of the progeny of the two parents with the F_1 and segregating generations of the crosses are highly desirable when genetic studies are to be made.

Suneson (1937) found that chilling wheat plants for periods of 15 to 24 hr. at 27 to 36°F. resulted in a marked reduction of self-fertile florets through killing of the pollen. Different varieties varied in tolerance to chilling.

Since the glumes of self-unfruitful florets are held open by the lodicules, rapid application of the desired foreign pollen by dusting on the exposed stigmas was possible. Self-fertile florets tend to remain closed and can be rogued. This method of emasculation might be useful if large numbers of hybrid seeds were needed. If the female parent possesses a simple recessive character and the male the dominant allele, any plants from self-fertilized seed can be rogued the year the F_1 plants are grown.

Rye.—In self-pollinating rye, several heads of a plant may be enclosed in a parchment bag before anthesis. It is desirable to place an eyelet in the top of the bag and to tie it to a stake for support. Bags of the same size as those used for ear bags with corn are satisfactory. These bags are left on the plants until harvest. In making controlled crosses between individual plants, the same technic used in emasculation and pollination with wheat, oats, or barley may be used. In making inbred-variety crosses, the flowers of the inbred lines may be emasculated in the usual manner, and when the florets open 1 to 4 days later a large amount of pollen may be collected by enclosing large numbers of heads of the open-pollinated variety in a large paper bag and the pollen applied to the emasculated flowers with a camel's-hair brush.

Flax.—Emasculations of flax are commonly made in mid-to-late afternoon. At this time a little experience will indicate which flowers will open the following morning. The petals of the flowers may be pulled out and the anthers pushed off with a toothpick. The next morning, flowers are collected from the male plants, and, by holding them between the thumb and forefinger, the dehiscing anthers may be brushed over the stigma. It does not appear to be necessary to bag the flowers.

Cotton.—In hybridizing cotton, it has been found that a short section of ordinary soda-fountain straw, closed at the upper end,

may be used to enclose the exposed pistil after emasculation. Humphrey and Tuller (1938) described an improvement in the use of this technic. They found it unnecessary to remove all the anthers in emasculation, since those not removed were cut off by the straw when this was inserted over the staminal column. The soda straw, before use, was closed at one end and about one-fourth of the anthers from a male flower scooped into the straw. This was then inserted over the pistil of the emasculated flower, forced down until it reached the ovary, and the straw fastened to the stem with No. 26 copper wire. By the use of this technic the flowers were emasculated and pollinated the same day, the flowers being worked with only once.

Sorghum.—Stephens and Quinby (1933) suggested the use of hot water for bulk emasculation of sorghum. If large amounts of hybrid seed were needed or backcross populations desirable, the ordinary methods of individual floret emasculation were too slow. They found that immersion of the sorghum heads in water, the temperature of which was 42 to 48°C., for 10 min. resulted in killing of the pollen. The equipment consisted of a large rubber tube that could be placed over the head to be treated and tied to the peduncle of the head at the lower end. A metal container was connected to the upper part of the tube, into which the hot water was poured. When proper temperature conditions were obtained, all pollen in the head was killed. The emasculated heads could then be pollinated with pollen of the desired male parent.

Rice.—Jodon (1938) found that immersion of the heads of rice in water at 40 to 44°C. for 10 min. destroyed the viability of pollen without injury to other floral organs. Treatment at 0 to 6°C. gave similar but probably less effective results.

A large-mouthed Thermos jug was used as a water container and the treatment applied in the morning prior to normal blooming. Emasculation by hot or cold water eliminated injury to the glumes, and the florets opened in a normal manner. Normal seed, which germinated well, was obtained when florets so emasculated were pollinated.

Another method used in artificial hybridization is to emasculate by removal of the anthers with small forceps through a slanting opening made by clipping away a portion of the upper part of the lemma. This is done in the evening or morning

prior to blooming, before the anthers will shed pollen on handling. The florets are pollinated by breaking mature anthers within the emasculated floret.

Potato.—For careful genetic experiments it is probably wise to enclose the flower clusters in small cloth bags in selfing the potato. Otherwise, bagging seems unnecessary. Emasculation is accomplished by removing the anthers with a small forceps or by scraping off the anthers with a small knife. Pollination is accomplished by tapping a flower of the male parent gently so that pollen is spilled onto the thumbnail and then applied to the stigma of the emasculated flower.

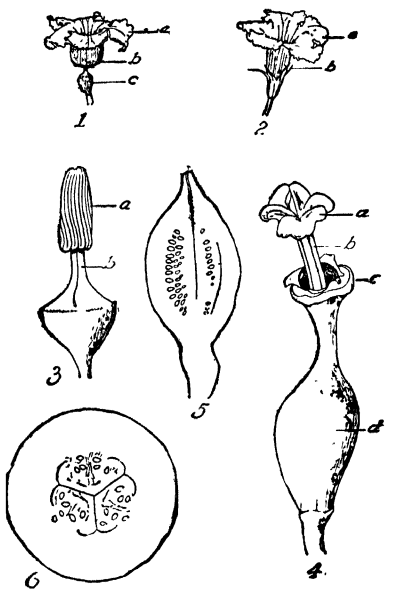


FIG. 9.—Structure of squash flower.
1. Female flower: (*a*) corolla; (*b*) calyx; (*c*) fruit.
2. Male flower.
3. Male flower with calyx and corolla removed.
4. Female flower with calyx and corolla removed showing: (*a*) stigma; (*b*) style; (*c*) point of attachment of calyx and corolla; (*d*) undeveloped fruit.
5, 6. Longitudinal and cross sections of fruit.

Size: 1, 2, ¼ ×; 3, 4, ½ ×; 5, 6 greatly reduced. (*After Hayes and Garber.*)

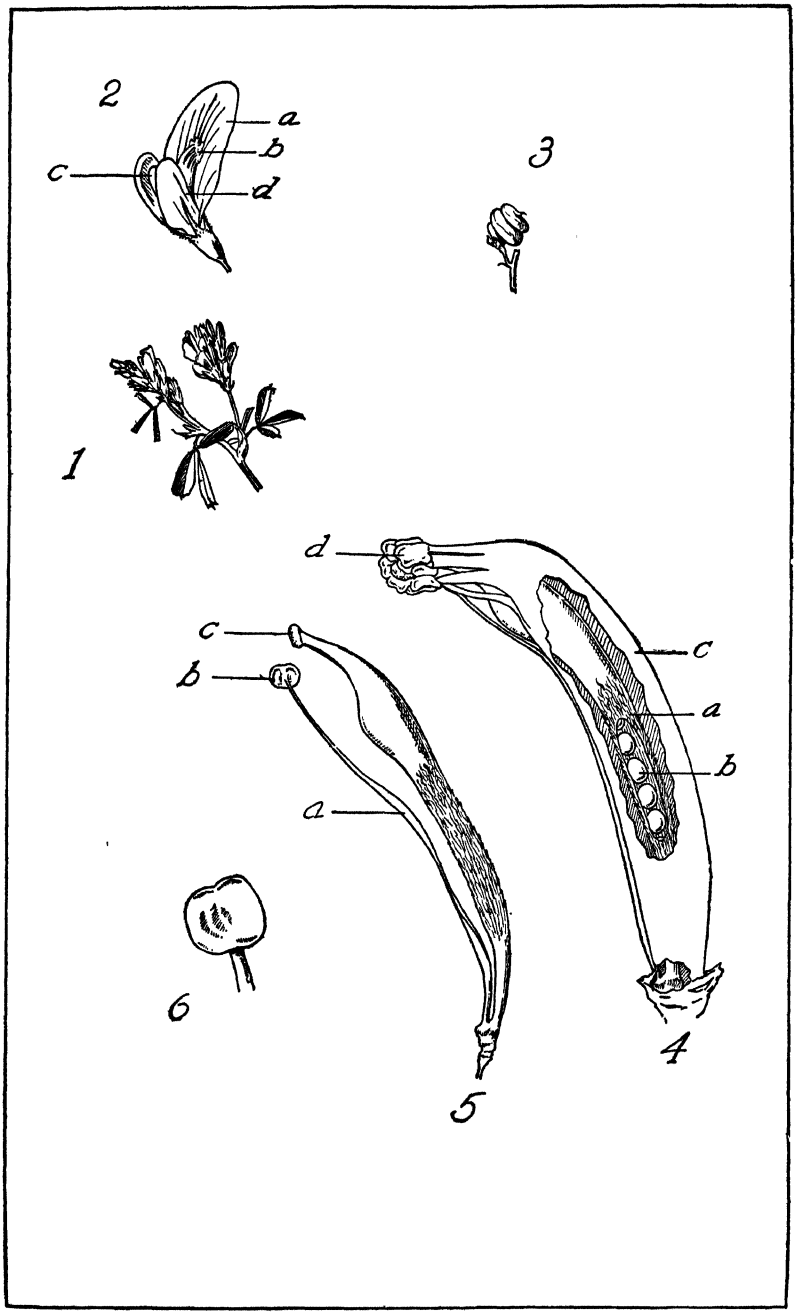
Pumpkin and Squash.—Most varieties of pumpkins and squashes have imperfect flowers, some flowers on the plant having only male and others only female organs. Pulling the petals of the female flowers together so that they completely cover the stigma and putting a rubber band around them are easy and acceptable ways of excluding foreign pollen. In selfing, or crossing, the male flowers may be collected and pollen shaken directly onto the stigma or first shaken onto the thumbnail and then transferred to the stigma.

Onion.—Onions may be selfed by enclosing the head with a paper bag before any pollen is shed. Shaking the plant daily or tying the bagged head to a stake so that the wind will do the shaking was found by Jones and Emsweller (1933) to increase the amount of seed set. For seed increases, large cheesecloth

cages may be used, several plants being enclosed. The authors mentioned above used cloth cages 3 by 3 by 6 ft. in making crosses. Fly pupae were introduced into the cages to act as a means of transferring pollen from one plant to another. Hybrids of some crosses may be distinguished in the seedling stage. In other crosses, it was necessary to grow the bulbs before roguing out the selfed plants. If the hybrids are not distinctly different from the female parent, it would be necessary to emasculate the female-parent flowers.

Red Clover.—In selfing red clover, cloth bags about 4 in. long and 2 in. wide may be used to exclude insects. Such bags are tied on before the flowers open. Usually a higher amount of selfed seed is obtained if the heads are rolled several times during the flowering period to aid in tripping the flowers. Cloth bags are preferred to paper, since the heads can be rolled without removing the bag. These bags may be made of theatrical gauze, such bags having a coarse mesh. Controlled cross-pollinations may be made by hand or through the use of bees. For hand pollination, Williams (1931*c*) states that emasculation is not necessary, since most red-clover plants are self-incompatible. Under Welsh conditions, the percentage of self-fertility varied from 3.45 to 0.17 per cent, with a mean of 0.85 per cent, during an 8-year period. Partly folded triangular pieces of cardboard 2 in. long and about ½ in. wide at the broad end and tapering to a point at the other are used for tripping the flowers and applying pollen. One card is used for tripping the flowers and the other for collecting pollen from the male parents and applying to the stigmas of the female parents. One collection of pollen usually will pollinate from 15 to 25 florets. After pollination, the pollinated heads are enclosed in cloth bags.

In using bumblebees for cross-pollination of red clover, the plants to be crossed are grown in pots. The parents to be crossed may be placed in insect-proof compartments in a greenhouse or in the field, the former being the more satisfactory. The bees are trapped in large test tubes (Williams 1931) and washed for about ½ min. by partly filling the tubes with water and shaking, the water being changed several times. The water is then poured off and the tubes placed in a rack for 2 or 3 min., after which the bees are rinsed two or three times before being placed in a wooden box to dry.



For descriptive legend see page 69.

In making paired crosses, the bees are introduced into a compartment containing the plants in about the half-bloom stage. After 4 to 7 days, the bees are removed, washed, and used for other crosses. When mass crosses are made, one bee to each six or eight plants is introduced into the compartment containing the plants and left until the flowering period is over. Similar methods have been found by Atwood (1940) to be satisfactory with white clover.

Alfalfa and Sweet Clover.—Several immature flowering branches of sweet clover may be covered with a lightweight checsecloth bag approximately 6 or 8 in. wide and 12 in. long. This excludes bees and prevents cross-pollination. There are three types of plants of *Melilotus alba*, according to Kirk and Stevenson: (1) those that are spontaneously self-pollinated and self-fertile and produce seed without manipulation; (2) those that are self-fertile but that are not normally self-pollinated without manipulation; (3) self-sterile plants. To ensure self-pollination, when the plant is self-fertile, it is desirable to manipulate the bag by rubbing gently with the hands every day or two at the time of pollination. *M. officinalis* is not entirely self-sterile, and selfed seed can be obtained by the same means (Pieters and Hollowell, 1937).

Kirk (1930) devised the suction method for emasculating sweet-clover flowers. If the plants are located near a water faucet, a vacuum flask inserted in the hose line will furnish the necessary suction. Otherwise, an electric or gasoline-driven suction pump must be used. A short piece of glass tubing slightly less than 1 mm. in diameter is inserted in the end of the hose. The point of this nozzle must be smooth, in order not to injure the flower. The amount of suction is of considerable

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FIG. 10.—Structure of alfalfa flowers.

1. Branch showing flowers in position.
2. Single flower, showing—*a*, standard; *b*, sexual column in contact with standard; *c*, keel; *d*, wings.
3. Seed pod.
4. Flower parts in position—*a*, undeveloped pod; *b*, ovary; *c*, filament; *d*, anther.
5. Same with all anthers removed except one to show stigma.
6. Anther.

Size: 1, about ½ ×; 2, about 2 ×; 3, about ½ ×; 4, 5, 6, greatly enlarged.

importance. All the flowers on a raceme are removed except about 20 per cent of the flowers that have most recently opened. The petals are next removed with forceps. This ruptures the stamens and scatters the pollen. With the use of the nozzle attached to the suction flask or pump, the anthers and adhering pollen are sucked off. The stamens should be approached from the side of the staminal tube in order not to draw the pistil and staminal tube into the end of the nozzle. After the stamens have been removed, the end of the nozzle should be passed over the surface of each style and stigma, sepals and axis of the rachis. If the operator wears a low-powered binocular magnifier on his head, he can check on the thoroughness of the emasculation while leaving his hands free. The pollen may be applied effectively with the end of the thumbnail.

Kirk found the degree of effectiveness of emasculation by suction to be 87 per cent but suggested that improvement in technic might increase this materially. He used the suction method on alfalfa also with good results.

Tysdal and Garl (1938) found that when suction alone was used and no foreign pollen applied to the stigmas, 14.1 per cent of the flowers formed pods. If suction plus washing with a stream of water was used, the percentage of flowers forming pods without application of foreign pollen was reduced to 5.5.

Tysdal suggested the use of alcohol as an agent for killing the pollen on the flowers of the female plants. The standards were first clipped from flowers in full bloom with a sharp scissors and the flowers tripped, leaving the stigmatic column exposed for treatment. All flowers on a raceme to be emasculated were treated in a similar manner. The raceme was then immersed for 10 min. in a beaker containing 57 per cent ethyl alcohol. The raceme was rinsed for a few seconds in another beaker containing water, after which the adhering water was blown off the stigma with a dentist's syringe or bulb from an atomizer and pollinated with the desired pollen. By the use of this method, the percentage of flowers forming pods without application of foreign pollen was 0.89. The percentage of flowers forming pods when foreign pollen was added was 26.3, as compared with 60.0 for the suction method. Emasculation by the use of alcohol was more complete, faster, and simpler than emasculating by suction.

Grasses.—Selfing, in greenhouses, may be accomplished by enclosing a number of inflorescences in a paper bag prior to pollen shedding. Bagging should be done soon enough so that stray pollen that fell on the flowering panicle or spikes before bagging will not remain viable long enough to effect cross-fertilization. Glassine or vegetable-parchment-paper bags are satisfactory. In closing the mouth of the bag, the stems are wrapped with cotton and the bag tied over this cotton. This excludes insects, if present, and helps to protect the stems from injury. The upper part of the bag is tied to a stake by means of a string inserted through an eyelet.

Bagging in the open requires careful consideration of possible damage due to wind and rain, as well as complete exclusion of



FIG. 11.—Studies of the effects of self-fertilization with grasses at the U.S. Department of Agriculture Regional Pasture Research Laboratory, State College, Pennsylvania.

foreign pollen. At Minnesota, vegetable-parchment-paper bags 4 by 2½ by 18 in., with round bottoms, sealed with casein glue, are used for the larger grasses, such as species of *Dactylis*, *Bromus*, *Phleum*, *Festuca*, *Agropyron*, and *Alopecurus*. A number of inflorescences are enclosed in a single bag. The leaves on the upper part of the stems are removed, cotton is placed around the stems, and the bags are tied around the cotton pad. The bottom end of the bag is tied loosely to a stake and the upper end tied tightly to the same stake, a string inserted through an eyelet put in with a small eyelet machine holding the bag at the upper end. Such bags allow for elongation of the stems and inflorescences. The bags are left on until harvest.

At the Welsh Plant Breeding Station, vegetable parchment that took the form of a sleeve (topless and bottomless) fitted

over a wire spiral was used. The wire spiral provides protection against storms. This sleeve was fitted over the inflorescences and tied to a stake at both top and bottom, a wad of cotton being first wrapped around the stake and the bag tied over this.

Jenkin (1931) reported that cotton sleeves (seamless), about 15 in. in diameter and from 3 to 4 ft. long, stretched over a frame, have been found to be highly effective in excluding foreign pollen, provided the proper type of fabric is used. The fabric found to be most satisfactory was a very dense and rather heavy fabric, the most closely woven that it was possible to procure. This fabric proved highly effective in excluding foreign pollen but was not absolutely pollen-proof. Cheesecloth gave very little or no protection. The cotton sleeve was tied to a stake in a manner similar to the method of fastening vegetable-parchment sleeves described above.

Kirk (1927) enclosed entire plants of brome grass in cotton cages about 5½ ft. high and 3½ ft. square as a means of effecting self-pollination. The bottom of the cotton cage was soaked in oil and buried a few inches in the soil. The tops were tightly tied. Foreign pollen probably was not absolutely excluded, but the method was highly effective when closely woven cloth was used. If all plants in the nursery not covered by cages were cut off prior to pollen shedding, it would be necessary for pollen to blow out through the cloth of one cage and in through the cloth of another and onto the flowers before crossing could be obtained. The amount of such cross-fertilization probably is very small.

In making crosses by hand-hybridization, Jenkin (1924) grew the plants to be crossed in pots and placed these in a cool greenhouse some time before flowering. Emasculation was done a few days before flowering. The upper and lower spikelets of an inflorescence were removed and the anthers removed from the remainder with a flat-pointed, blunt pair of forceps, the upper florets being emasculated first. In *Phleum*, *Alopecurus*, and *Phalaris*, severe thinning of the florets in an inflorescence is necessary. After emasculation, the inflorescence is covered with a paper bag.

Inflorescences of the male parents are covered with paper bags prior to flowering. The greenhouse is closed tightly about an hour before pollination begins so that any floating pollen may

settle down. The bag on the male plant is inclined so that when shaken vigorously the pollen collects in the creases toward the mouth. The bag is removed and the pollen poured on a sheet of dark, glossy paper, previously folded into a boat shape and cut with a sharp point at one end. The pollen is brushed lightly over the stigmas of the emasculated flowers and the female unit rebagged. Since all florets do not open on the same day and flowering proceeds progressively downward, pollination is repeated every day until no more fresh stigmas are produced. Jenkin (1931*c*) reported successful crosses, by the foregoing method, with species of *Lolium*, *Festuca*, *Arrhenatherum*, *Dactylis*, *Phleum*, and *Alopecurus*.

CHAPTER V

THE PURE-LINE METHOD OF BREEDING NATURALLY SELF-POLLINATED PLANTS

EARLY STUDIES

This method has been developed as a result of fundamental studies like those of Vilmorin, Mendel, Johannsen, and of numerous workers in recent times. These studies, together with field experience, have led to the conclusion that the progeny of an individual plant selection with self-pollinated crops may be expected, for the most part, to breed true immediately.

A brief review of some of the more important of these early studies will be of interest.

Le Couteur, in the early part of the nineteenth century, was a farmer on the isle of Jersey who was interested in the problem of improving his crops. Professor La Gaska, from the University of Madrid, visited Le Couteur and pointed out numerous differences in plant type occurring in Le Couteur's wheat field. Selections were made and the progenies tested. Some proved superior to the commercial variety and were of more uniform habit of growth; other selections were of little value. Bellevue de Talevera, one of these selections, was of commercial value for many years.

Patrick Shirreff, a Scotsman, carried on selection with wheat and oats at about the same period as Le Couteur. He used the individual-plant method, selecting strong, vigorous plants in his wheat and oat fields, keeping the progeny of individual plants separate, and increasing the more desirable. Like Le Couteur, he proceeded on the assumption that the selected single plants would breed true. New varieties produced by this means were grown extensively.

Hallett began selection with wheat, oats, and barley about 1857, believing, apparently, that acquired characters were inherited and that improvement induced by favorable growing conditions would be transmitted to the progeny. He raised his plants, therefore, under the most favorable cultural conditions,

selecting the best seed on the best developed head of the more vigorous plants, replanting, and following the same plan of selection in subsequent years. New varieties were introduced, the best known being Chevalier barley. Although the method appears less desirable than that of Shirreff and there is little reason to suppose that the continuous selection was of value in isolating new heritable variations, it gave an opportunity to study progenies during different seasons and in this way to select the best. New varieties were introduced that proved of value.

The Vilmorins, in France, were early leaders in improvement of plants by selection. Louis de Vilmorin (1856) developed the progeny test with reference to sugar beets. Early wheat selections were made also, and the method developed is known as Vilmorin's isolation principle. Briefly, this consists of the fact, well known today, that the only sure means of knowing the value of an individual-plant selection is to grow and examine its progeny. Methods were developed for the determination of the sugar content of individual roots of sugar beets. Louis de Vilmorin observed that the progeny of some beets of high sugar content gave progenies of high sugar content rather uniformly, whereas others gave progeny of both high and low content and still others gave progeny that were uniformly of low sugar content. Four varieties of wheat were propagated for 50 years by selecting the best plants each year. At the end of the selection period they were compared with specimens saved at the beginning of the experiment, and no change was noted.

Newman (1912) made an interesting review of plant breeding in Scandinavia. The Swedish Seed Association, formed in 1886, had a marked influence on the development of plant-breeding methods. Hjalmar Nilsson became the director of the association in 1891. From the beginning, careful records were kept, individual plants were classified on the basis of minute botanical differences, and seed of plants containing the same characteristics was combined, the progeny of each separate type being grown in a separate plot. Some progenies appeared so uniform that they were especially noted by Nilsson. From a study of the records, it was learned that in each case these were from seed of an individual plant, there being only one representative of that morphological group. This led, naturally, to the individual-plant method of selection.

W. M. Hays started his plant-breeding program in Minnesota in 1888 and from the beginning used the individual-plant method of selection. Besides making practical studies, he initiated many experiments that had as their purpose the formulation of fundamental principles. He developed the centgener plan of plant breeding. The first step consisted of selecting individual plants of promise, threshing these separately, and making nursery trials of their progeny. During the period of study, plots of 100 plants each were grown from each selection. Besides taking notes on yield and other characters on the plot basis, the 10 better plants in each plot were selected in the field, threshed individually, and the seed of the 5 that were of greatest promise, after laboratory study, was bulked and used for the following year's centgener plot. One of the difficulties of the method as a yield trial was that when numerous selections were made it took several days to plant the nursery and, since only one plot of each selection was grown, as a rule, the data obtained were not comparable. The types of greatest promise, however, were quickly isolated and grown in larger plots. Improved Fife, Minnesota 163, and Haynes Bluestem, Minnesota 169, were valuable new varieties of spring wheat selected by this method and grown widely in the early part of the present century.

THE PURE-LINE THEORY

The experiences of plant breeders played their part in developing breeding methods, but it remained for Johannsen to place the individual-plant method of selection on a firm scientific basis.

Johannsen (1903, 1909) made his studies with beans, selecting this plant because it belongs to the self-pollinated group and contains characters that are easy to measure. He hoped to control heredity by applying Galton's law of regression, *i.e.*, that the progeny of parents above or below the average tend to revert to the average type. The tendency to regression toward the average could be measured and expressed statistically. By selecting extreme parents, continual improvement could be obtained, if the same degree of inheritance was obtained in later generations. In studying size of beans, Johannsen found a different regression value from that obtained by Galton and less progressive improvement than he expected. This led to a study of the progeny of individual plants, each of which varied around its mean. He

found each of these progenies to be a single hereditary line, within which there was complete regression to the mean of the line when extreme parents were selected and their progeny studied. These principles are well understood today and have had a profound effect on plant-breeding practices. Johannsen defined a pure line as the descendants of a single, homozygous, self-fertilized organism. Jones gave a definition, which is in common use today, by stating that a pure line comprises the descendants of one or more individuals of like germinal constitution that have undergone no germinal change.

THE PURE-LINE THEORY IN ITS APPLICATION

Although many experiments have been carried out that prove that continuous selection in self-pollinated crops, as a means of obtaining further improvement, is not worth while and there is general appreciation of the fact that the initial individual selection is of greatest importance, there is a growing body of evidence that heritable variations are more frequent than was supposed at one time. An illustration may be useful. Several years ago, Victory oats, originally produced in Sweden from an individual-plant selection, was on the recommended list of the Minnesota Agricultural Experiment Station. A large number of individual-plant selections were made and their progeny studied by R. J. Garber. When these various lines were compared for differences of plant-breeding importance, in replicated rod-row trials, no new line was obtained that was appreciably superior to the commercial seed of Victory that had been distributed to Minnesota farmers. The different selections showed, however, numerous minor, heritable differences of distinct morphological type as well as differences in quantitative characters that were more difficult to evaluate exactly.

In this connection, recent papers by East (1935*a*,*b*,*c*, 1936*a*) deserve consideration. He classified gene mutations under two categories: "physiological defectives" and nondefective genes. The former are the genes that have been used, largely, in genetic experiments. An illustration may be given of East's viewpoint by reference to the ligule that is characteristic of the entire group of *Gramineae*. Liguleless stocks are known in maize, rye, wheat, and oats, and in some cases the difference between liguled

and liguleless types behaves as if controlled by a single factor pair. East suggests that the ligule "presumably is the result of a very large number of non-defective mutations in various genes, and, physiologically speaking, is the end product of a long chain of reactions." A single mutation breaks this chain, and a liguleless plant results.

East states that he believes every experienced plant breeder will agree with the statement that "non-defective gene mutations are frequent in Nature, but are difficult to detect." He summarizes the results with tobacco, where characters of plants in self-fertilized lines were evaluated statistically. Although there was a rapid approach toward uniformity and gross homozygosis, there still remained considerable variability, a part of which was proved to be heritable. High mutation frequently is believed to be responsible for heritable changes in these small genetic factors of the nondefective type.

East gives an illustration of several cases in which, in attempts to produce certain species hybrids, only maternals resulted. The plants were ordinary fertile diploids and, presumably, arose from mature gametes in which parthenogenesis was induced. The plants then would be completely homozygous. In cases in *Nicotiana rustica*, each progeny row was astonishingly alike, more so than "any ordinary inbred populations that I had ever examined." Several of these lines were continued by self-fertilization and within 3 or 4 years were as variable as ordinary inbred populations.

For practical purposes, the pure-line theory furnishes a basis for the isolation of types that differ appreciably in heritable characters, and the progeny of individual plants in self-pollinated crops breed relatively true. Mutations do occur, and minor mutations of a nondefective type are relatively frequent, although often not sufficiently large to be of major selection value.

Natural crosses are more frequent than is generally appreciated and furnish another basis for variation among plants within a variety or strain. Mechanical mixtures occur also. These various causes emphasize the necessity of constant care to ensure the necessary uniformity desired in an improved variety. They do not detract from the value of the pure-line concept in its application to the improvement of self-pollinated crops by individual-plant selection.

METHODS OF IMPROVING SELF-FERTILIZED PLANTS BY INDIVIDUAL-PLANT SELECTION

The following condensed summary of methods will serve as a basis for a plan with particular crops. It is stated in general terms, for it is recognized that such widely different plants as tomatoes, tobacco, rice, and wheat must be grown according to their special adaptations. With crops such as tobacco and tomatoes, individual plants will be separately spaced in rows or plots, whereas with the small grains bulk sowing of seed may be practiced from the beginning of the trials.

Two principal sources of selections are available in the production of new varieties:

1. The introduction of improved or relatively unimproved strains and varieties of crops found in use over a wide range of conditions, both foreign and domestic.

2. Well-adapted local varieties that are found to be variable and to contain a composite of a number of biotypes. These may have had their origin as hybrids or pure lines, which have become altered as to general type through mechanical mixtures, natural crossing, or mutations.

I. Utilization of Introductions

A. Source of materials.

1. A list of introduced crops and varieties with their descriptions may be obtained from the U.S. Department of Agriculture, Bureau of Plant Industry. It will be desirable to determine through the bureau, when possible, the adaptation range of the introductions that appear to meet the needs and secure seed from this source.
2. Personal contacts with foreign and domestic visitors is a natural source of introductions for crops that are developed along special lines but that frequently do not arrive through the channels of the Bureau of Plant Industry. The visits of staff members to foreign and domestic stations likewise may occasionally bring to attention special-purpose crops and their varieties.
3. Mutual exchange of crops with domestic stations is a desirable practice. Station publications afford a description of crops in use.
4. A survey of farm varieties is desirable. Native species, especially with forage crops, may yield a source of new material.

B. History and records of all introductions.

It will be desirable to keep a record book or card file recording as follows:

1. History of each introduction.
2. Description of same.
3. Year introduced.

C. System of records.

A system of numbering new varieties that ensures ease of interpretation and accuracy of record is desirable.

1. The Minnesota method is presented here and, for comparison, notations for introductions, selections, and hybrids are included.

Minnesota Records

I-20-1	Selections
II-20-1	Crosses
III-20-1	New introductions

In this method, I, II, and III stand for individual-plant selections, crosses, and introductions, respectively; 20 represents the year in which the selection, cross, or introduction was made; and the final number represents the particular selection, cross, or introduction. Crosses are given a selection number only after having been shown to be homozygous. Parental and F_n populations are numbered by carrying row numbers for the current and preceding season, until homozygosity is reached. When the method of carrying row numbers for 2 years is used in the planting plan, a pedigree can be completed when desired. The method often used by workers in the U.S. Department of Agriculture or in state experiment stations is given here where F_1 = A, F_2 = A-1, A-2, etc., F_3 = A-1-1, A-1-2, etc., according to the number of selections grown.

First year = II-18, A.
Second year = II-18, A-1, A-2, etc.
Third year = II-18, A-1-1, A-1-2, etc.

Selections of crosses, when given series numbers, after reaching homozygosity, are designated II-18-1, II-18-2, etc., according to the number of selections made.

2. Alberta system—modification of method 1.

I = introduction.
S = selection.
H = hybrid.

Otherwise the method of numbering is similar to that used in Minnesota.

D. Observation of introductions.

1. All introductions may be placed under observation in small plantings. Some of the original seed should be retained in case of unfavorable growing conditions the first season and for later comparison in the case of selection.
   a. Plots will consist of single short rows for small grains; other types of small plots may be used for other crops.
   b. The first observations will be concerned especially with characters of outstanding known value or for adaptability, uniformity, and general utility of the crop. The new introductions will be compared with standard varieties that are sown as frequent checks in these observation tests.

c. This preliminary planting may serve also as a means of seed increase for larger trials.

2. During the second year, observations will again be based on small plantings similar to those of the first year and may serve as a natural means of eliminating those that are poorly adapted. These second plantings are also a means of seed increase.

E. Method of testing desirable introductions for further trial (Love and Craig 1918*a*, 1924) (Noll 1927) (Goulden 1931).

By means of several years of observation, a few introductions may have been found that appear to fill a special need. The procedure of testing these is outlined under II *C*.

II. Pedigree Selection within Adapted Varieties

A. Agronomic characters sought according to needs of the regions concerned.

Some important agronomic characters are given below:

1. Small grains and other cereals.

Winter hardiness	Awn characters (barley)
Straw strength	Presence and absence of awns
Time of maturity	Percentage of hull (barley and oats)
Drought resistance	Seed color
Quality	Yielding ability
Nonshattering habit	

2. Forage crops.

Growth habit	Leafiness
Quality	Recovery after grazing or cutting
Drought resistance	Cold resistance
Straw strength	Yielding ability (forage and seed)
Contribution to soil organic content	Palatability
	Nutritive value

3. Root, tuber, and sugar crops.

Sugar content	Palatability
Ratio of roots to tops	Quality
Nutritive value	Yielding ability
Seed production	Frost resistance

Note: This list is not intended to be exhaustive and may be supplemented according to the interests of the individual.

B. Resistance to diseases and insects.

1. Selection of strains resistant to diseases that are difficult to control except through the production of resistant varieties is of greatest importance. The following diseases may be mentioned:

Rusts	Blights	Root and stalk rots
Smuts	Wilts	Take-all
Mosaic	Scab	Anthracnose

2. Selection of pathogens.

a. Study the disease reaction in special disease nurseries and in the greenhouse with the use of physiologic races existing in the particular locality or over a wider region.

b. Grow special disease nurseries in several places in the area in order to test for resistance under field conditions to physiologic races as they occur naturally.

3. Disease garden.
   a. Selections should be tested in short rows or in other types of plots.
   b. Disease epidemics.
      (1) Artificial epidemics should be induced on susceptible border rows grown throughout the nursery and generously distributed through the plots of tested varieties or directly on the varieties themselves.
      (2) Natural or artificial epidemics may be obtained by growing the particular crops on soil infected by wilt, root rot, etc.
4. Insect pests.
   a. Grow short-row plots on soil or in regions infested with such insects as Hessian fly, jointworms, boll weevils, borers, etc.
5. Replication frequently is important in testing for resistance to plant diseases or insect pests. It is helpful in many cases to plant at different periods in order to obtain favorable conditions for producing the epidemic.

C. Technic for selection and testing.

1. Single-plant basis for selecting lines.
   a. First year. Select approximately 1000 heads from individual plants of the type desired. The total number of initial selections depends on the crop and the amount of land and funds available for subsequent testing.
   b. Second year. Sow 25 to 50 seeds of each selected plant in space- or bulk-planted single-plant or head-progeny rows. Discard all plant rows that appear of undesirable type. Heterozygous types of extreme promise may be reselected. Continue elimination of undesirable types in each successive year. Bulk seed of the individuals for test in progeny rows.
   c. Third year. Replication should be started this year for preliminary yield trials. Observe lines for uniformity for such agronomic characters as date of heading, strength of straw, and height of plant. Disease tests may be carried out as described under II *B* 3.
   d. Fourth to sixth years. The number of years indicated is arbitrarily suggested, and these trials should be continued to the extent found desirable.
      (1) Composite seed of the replications of previous year's test and grow in single- or three-row plots or in other types of plots when desired. Replication is necessary.
      (2) Plant a duplicate test in the disease garden each year, and test for resistance to important diseases and insect pests.
      (3) Test for special characters—grow replications in a particular environment, as on peat, sand, etc.
      (4) Make quality tests on the crop from border rows.
      (5) Select the more desirable lines for more extensive trials.

e. Seventh to ninth year. Test promising lines in advanced trials, *i.e.*, in $\frac{1}{40}$-acre plots replicated or in row plots with more replications than in earlier years and, when possible, at a number of stations. In these yield trials, replicate to the extent found necessary.

III. Cooperative Tests and Distribution of Promising Lines

A. Bring information of the proved lines and introductions before the farmers through the extension service, agricultural high-school teachers, county agents, and crop-improvement associations and by means of bulletins.

B. Select reliable farmers to grow demonstration plots of the improved lines in comparison with standard varieties.

1. Plots, a single drill width, in the center of the farmer's field are used in Minnesota.
2. A replicated trial may be made with a few farmers or local schools when desirable.

C. Arrange these demonstration projects in a number of counties or provinces, and organize a field day for the community at which the county agent can use these plots as part of his program.

D. Distribute seed to those interested through the farmers' crop-improvement association.

ILLUSTRATIONS OF VALUABLE VARIETIES OF SELF-POLLINATED PLANTS PRODUCED BY APPLICATION OF THE PURE-LINE THEORY

Selection has played a large part in the production of new varieties of wheat, oats, barley, flax, and other self-fertilized crop plants. Clark (1936) has given the origin of many of the varieties of spring and winter wheat. In winter wheat, Iobred, Ioturk, and Iowin, selected by L. C. Burnett, at Ames, Iowa, have been grown extensively. Nittany, selected from the Fulcaster variety by Noll, is the principal variety grown in Pennsylvania. Nebraska 60, selected by Kiesselbach from the Turkey variety, is grown widely in Nebraska, and Kanred, selected from Crimean by Roberts, with an estimated acreage of 3½ million acres in 1929, has been of great value in the hard red winter-wheat region.

In spring wheat, the early selections, Improved Fife (Minn. 163) and Haynes Bluestem (Minn. 169) introduced about 1900 were important varieties in the early part of the present century. Mindum *durum*, the standard for quality of semolina products and the most widely grown durum variety in United States and

Canada, was produced by plant selection at the Minnesota station.

In a discussion of superior germ plasm in oats, Stanton (1936) described many new varieties that have been developed by plant selection. Fulghum oats and its many strains originated from a single plant selected from Red Rustproof by J. A. Fulghum. The single plant was earlier and taller than the Red Rustproof variety. Other important selections from Red Rustproof and Fulghum include Kanota, Franklin, Columbia, Nortex, and Frazier.

Varieties of Kherson and Sixty-day oats are grown extensively in regions of the corn belt where early oats of the *Avena sativa* group seem desirable. Gopher, a white-seeded strain of sixty-day, has been grown extensively in southern Minnesota and in other states where early oats are adapted. It is perhaps the stiffest strawed early variety available. Its production emphasizes the ease of improvement in some cases. Only 200 original plant selections were made from an early variety with mixed seed color. The first year in plant rows, six strains excelled in strength of straw, and the remainder were immediately discarded. Gopher was the best yielding strain of the six. Richland and Iogold selected by Burnett are both resistant to black-stem rust. Nebraska 21, selected in Nebraska, has been grown widely. State Pride, a plant selection made in Wisconsin, has been the standard early variety in that state.

Among midseason varieties, Colorado 37 is outstanding in strength of straw and suitability for growing under irrigation. Cornellian, Ithacan, Upright, and Lenroc, selected by Love, in New York, occupy about 50 per cent of the oat acreage in that state. Rainbow and Rusota are important varieties selected from Green Russian at the North Dakota station. Both are resistant to black-stem rust.

Improved varieties of barley resulting from plant selection have been given by Harlan and Martini (1936). A few of the more widely grown varieties will be mentioned. Atlas selected from the coast variety is the most important variety in California. In the Manchuria-Oderbrucker group, Manchuria, Minn. 184, was selected at the Minnesota station. Wisconsin Pedigrees 5 and 6 selected from Oderbrucker are the chief strains selected from this variety. Peatland, selected at Minnesota, in cooperation

with Harlan of the U.S. Department of Agriculture, is especially well adapted to peat soils and is valuable also because of its resistance to scab and black stem rust. Trebi, selected by Harlan, was grown on an estimated acreage of 2,224,000 in 1935, the largest acreage devoted to any single variety. It is not a desirable malting variety, but in spite of several undesirable characters it has high yielding ability and is especially well adapted for growing under irrigation.

Practically all the varieties of rice grown in the United States, according to Jones (1936), were developed by selection, although not all were obtained by pure-line selection. More recently, hybridization has been used as a method of breeding, but to date only one variety produced by hybridization is grown commercially.

Dillman (1936) states that all varieties of seed flax grown in the United States were obtained by plant selection. Bison, selected by Bolley, at the North Dakota station, from commercial seed obtained from Belgium, is the most widely grown variety in United States. Buda, selected also by Bolley, has been a popular variety. Redwing, selected in Minnesota, is an early-maturing variety well adapted to southern Minnesota and Iowa, where it is extensively grown. All three varieties are resistant to wilt. Without this resistance it would have been impossible to continue to grow flax in the hard red spring-wheat belt.

Individual-plant selection has been of great importance also in peas and beans (Wade 1937). Strains of Alaska peas and of other varieties have been selected that are resistant to fusarium wilt. M.A.C. Robust, selected by Spragg, in Michigan, is resistant to mosaic and has been grown extensively in Michigan and New York. Among the present varieties of soybeans, Morse and Cartter (1937) state that a considerable proportion were obtained by selection from the large number of introductions that were obtained from the Orient. Individual-plant selection has played a large part also in the origin of tobacco varieties, as has been pointed out by Garner, Allard, and Clayton (1936).

CHAPTER VI

HYBRIDIZATION AS A METHOD OF IMPROVING SELF-FERTILIZED PLANTS

SOME STUDIES BEFORE 1900

Many studies were made during the eighteenth and nineteenth century for the purpose of learning the laws of inheritance in hybrids or to develop new and improved varieties. A few of the more important of these early studies will be mentioned to suggest the extent of the many investigations made before the present century, each of which played a part in developing principles that have led to the present view of a planned plant breeding program.

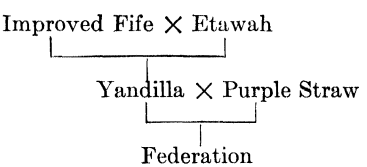
Kölreuter, in 1760–1766, made extensive studies of artificial hybrids and emphasized especially hybrid vigor in F_1 crosses. He noted the intermediate condition of the F_1 in crosses in tobacco and interpreted this as showing the effect of the male parent. Thomas Andrew Knight, born in England in 1759, contributed greatly to early plant breeding. Much of his work was with fruit crops—apples, pears, peaches, currants, and grapes. He emphasized the value of crosses as a means of obtaining new combinations of characters. John Goss, about 1820, studied segregation in crosses with peas but did not give an adequate explanation of the nature of the segregation. Sargaret, about the same period, made crosses between muskmelon and cantaloupe and studied fruit characters in F_1. He reported the appearance of differences in flesh colors, seed color, rough or smooth fruit, extent of ribbing and flavor and emphasized the dominance of one character over the other. Gärtner, in 1849, studied thousands of crosses, observing the uniformity and appearance of the F_1 generation. Naudin, in 1865, just prior to Mendel's report, noted the uniformity of the F_1 generation and segregation in F_2, ascribing this to the segregation of heritable factors in the formation of male and female reproductive gametes.

Mendel's work need be mentioned only briefly. He studied individual characters and placed his results on a definite factor

basis. The methods used by him are not widely different from those used today. Although the laws of inheritance are much more complex than those presented by Mendel and although most normal characters are dependent upon the interaction of many genetic factors, the methods of work introduced by him have found very wide application. These methods have made it possible to develop a planned plant-breeding program based on the laws of heredity.

William Farrer, of Australia, during the latter part of the nineteenth century, approached present-day plant-breeding methods and developed many wheat varieties of great value. He selected parents for crosses on the basis of their characters, strongly featuring the value of composite crosses as a means of inducing maximum variation. Federation, a variety of wheat that was early maturing, nonshattering, with stiff, erect, short straw, was produced as the result of a definite attempt to obtain a variety of wheat suited to gathering with a stripper.

An illustration of the crosses used in the parentage of Federation is given below:



By similar means, he obtained the following varieties: Comeback, Ceder, Firbank, Bobs, Cleveland, and Florence, the latter being a bunt-resistant variety.

The work of A. P. and C. P. Saunders in Canada is well known. In 1892, A. P. Saunders crossed Hard Red Calcutta with Red Fife. C. P. Saunders took over the experimental work at Ottawa in 1903 and continued the selections that led to Marquis and other varieties, the new variety Marquis being first grown in pure form in 1904, 12 years after the original cross was made. He used the individual-plant method of selection and determined the gluten quality in the progeny of crossbred wheats by the chewing test.

DEVELOPMENT OF METHODS SINCE 1900

The rediscovery of Mendel's laws by De Vries, Correns, and von Tschermak, in 1900, stimulated the extensive studies of the

laws of heredity that have led to the present system of breeding crop plants with a definite plan to obtain the combination of characters desired. The first step in such a program is a careful study of material available and an analysis of the characters desired. The necessity of making a collection of all available strains and varieties and an analysis of their characters needs emphasis. Although there is general appreciation of the desirability of this step, it is seldom practiced to the extent that would seem to be worth while. In recent years, Vavilov and his coworkers in Russia have made extensive world-wide collections of many crops. The U.S. Department of Agriculture maintains very extensive collections of varieties of grains, fruits, and vegetables that serve as a potential source of material for breeding. New strains and varieties are being added constantly through the plant-introduction service. The second step is to obtain the most desirable strains by selection. When these steps have been taken and the necessary background of knowledge with respect to disease reaction and agronomic characters of the crop has been gained, a crossing program may be undertaken. Crosses are made with a definite purpose in mind, *i.e.*, with the intention of combining in one variety the characters desired. Illustrations will be given from some of the present problems being studied at Minnesota.

BREEDING IMPROVED VARIETIES OF BARLEY

This work, cooperative between plant geneticists, plant pathologists, and cereal technologists, illustrates the value of a cooperative program.

The first crosses for the Minnesota experiments, designed to produce satisfactory smooth-awned barleys, were made in 1912. These involved crosses of Lion, a six-rowed, black-glumed, smooth-awned variety with good, adapted, six-rowed, white-glumed, rough-awned sorts. Strains with white glumes and smooth awns were selected and appeared to yield well in rod-row tests (Harlan and Hayes 1919). In more extensive tests (Hayes 1926), it was found that in some seasons these strains were reduced in yield because of the "spot-blotch" disease caused by *Helminthosporium sativum*. This led to a cooperative attack on the problem by plant geneticists and plant pathologists. It was

found that the greater susceptibility of the hybrids appeared to be due solely to their greater susceptibility to *H. sativum.*

A second series of crosses was made, with the use of one of the better white-grained, smooth-awned segregates of the first cross with desirable white-grained strains of the Manchuria type that were resistant to spot blotch.

Studies of disease resistance were made in specially prepared disease gardens. The mode of inheritance of reaction to Helminthosporium was studied in the early segregating generations (Hayes *et al.* 1923 and Griffee 1925), and it was found that at least three genetic factors were involved in differentiating reaction to the spot-blotch disease.

Selections of smooth-awned segregates of desirable plant and kernel type were made in the plant rows found to be resistant to *H. sativum.* From the cross of a smooth-awned segregate of the first cross with Luth was produced the variety Velvet. The cross of "smooth awn" × Manchuria led to the production of Glabron. Velvet is grown rather extensively at the present time. The most extensively grown smooth-awned variety of the Manchuria type is Barbless (Wisconsin 38) produced from a cross of Lion × Oderbrucker by Leith of the Wisconsin Agricultural Experiment Station.

The smooth-awned varieties Velvet, Glabron, and Barbless are susceptible to stem rust and to blight. The variety Peatland, selected at Minnesota from a lot of seed obtained from Switzerland, is resistant to both diseases. Studies by Powers and Hines (1933) and by Reid (1938) showed that the stem-rust resistance of Peatland was due to a single dominant factor, in crosses with susceptible varieties. Brookins (1940) has shown that the factor pair conditioning resistance and susceptibility to a collection of races of rust in the field in the mature-plant stage also controls the same type of reaction to races 19, 36, and 56 in the seedling stage in the greenhouse.

Peatland was crossed with Barbless for the purpose of combining the good characters of both. The contrasted characters are given in the table shown on page 90.

A large F_2 was grown in a space-planted nursery and only plants with smooth awns that were resistant to stem rust and that were of desirable plant and seed type were selected. Since smooth awns are due to one main recessive factor, the selection of smooth-

awned plants in F_2 eliminated all rough-awned sorts from subsequent generations. Resistance to stem rust is dominant. Consequently, the F_2 resistant plants gave rise to homozygous and heterozygous F_3 progenies in a ratio of 1:2.

Character	Barbless	Peatland
Yield	Good*	Fair
Seed size	Large*	Moderately small
Type of awns	Smooth*	Rough
Strength of straw	Poor	Fair*
Resistance to stem rust	Susceptible	Resistant*
Resistance to scab and blight	Susceptible	Resistant*
Resistance to loose smut	Susceptible	Resistant*
Resistance to covered smut	Moderately resistant*	Resistant*
Resistance to stripe	Moderately resistant*	Susceptible
Resistance to spot blotch	Moderately resistant*	Moderately resistant*

* Indicates desired character.

Tests of reaction to blight were begun in F_3 or F_4 in a special tent under conditions conducive to the development of a severe epidemic. Notes on reaction to blight are taken just before the heads ripen. These notes are then used in conjunction with notes on reaction to stem rust and smut, date of heading, height of plants, and lodging in conjunction with observation of general vigor and appearance of the plants in making individual-plant selections. Many of these strains are now in rod-row trials and appear promising.

Other crosses are, of course, made also and carried along in a parallel manner. One of these involves Velvet × Chevron. Chevron is a sister selection of Peatland and has a similar reaction to the common barley diseases. If some of the best segregates are found to be a distinct improvement over the existing varieties, they will be released for distribution to the farmers. The best of these, from different crosses, may then be crossed in an effort to bring about further improvement.

Tests of yielding ability begin with rod-row trials at University Farm only. The best of these are then tested in replicated yield trials in four stations in Minnesota. After a 3-year test in rod rows, the most promising strains are then tested in $\frac{1}{40}$-acre-plot

trials in six places in Minnesota, usually for a period of 3 years, before a final conclusion regarding distribution to the growers is made.

Studies of diastatic activity are made on the material in rod rows in cooperation with the Division of Agricultural Biochemistry at the University of Minnesota. When the new strains go into $\frac{1}{40}$-acre-plot trials, a complete malting test is made in the cooperative malting laboratory at the University of Wisconsin.

BREEDING BY HYBRIDIZATION

Examples have been given above and in previous chapters of a few problems that are being attacked or that have been solved through the crossing of different varieties and the combination of characters from them. The broad principles involved in a hybridization program, the selection of the parental material, and a general description of the method of handling the hybrid material will be discussed here. The detailed outline of the steps to be followed in successive generations following the cross will be given under Methods of Breeding.

Object of Crossing.—The object of crossing is to combine in a single variety the desirable characters of two or more lines, varieties, or species. Occasionally the recombination of genetic factors leads to the production of new and desirable characters not found in either parent. In a planned program every effort should be made, however, to select parents that have the characters desired. Frequently transgressive segregation occurs for quantitative characters such as yield, height of plant, earliness, and resistance to lodging. Selection of parents that are already relatively satisfactory for these characters will enhance the probability of obtaining the desired end result.

Selection of Parental Material.—The procedure to be followed in selecting parental material for crosses will depend upon the extent to which the station conducting the breeding program has previously experimented with the crop in question. A station that has conducted extensive variety tests for any given crop will in all probability have sufficient data to inaugurate a breeding program without further study of the parental material. However, a station beginning a breeding program with a crop of which it knows relatively little should conduct a thorough study of all present varieties (and of species in some cases) of that crop before



FIG. 12.—Uton oats was bred in Utah by Tingey, Woodward, and Stanton (1941). It combines the large, white kernel of its Swedish Select parent with resistance to smuts from its Markton parent.

The upper photograph shows reaction to loose smut and the lower photograph to covered smut. The two bundles at the left of the photographs show the proportion of smutted and smut-free panicles of Swedish Select, the two at the right the smutted and smut-free panicles of Uton.

beginning a breeding program. The importance of having a thorough knowledge of the parental material cannot be too strongly emphasized.

Technique of Crossing.—Crossing may be performed in either greenhouse or field. The greenhouse offers better protection from the elements and from stray pollen and often provides more satisfactory temperature and humidity. Crosses in the greenhouse can be made at almost any time of the year.

It is advisable to make several dates of planting, particularly when the parents differ in time of flowering. The parents should be sown in short rows (or in pots), with the seeds individually spaced and with sufficient space between the rows so that the plants can be worked with easily.

A study of the structure of the flower will reveal the best method of making the crosses. A study of the viability of the pollen and the time of receptivity of the stigma will be of material aid. An examination of the stigma and anthers sometimes will reveal which of the two parents should be used as the female. For example, it is known that it is more difficult to obtain crossed seed when barley varieties with unbranched stigmas are used as females than when varieties with branched stigmas are used as females. It is important also to prevent as much injury to the flowers as possible. If possible, use as the female the parent with a recessive character so that selfs can be discarded when the F_1 is grown. The details of emasculation and pollination were discussed in Chap. IV.

Handling the Hybrid Material.—Sufficient F_1 plants are necessary to give the amount of seed required for the F_2. If grown in the field, the F_1 seeds should be individually space-planted far enough apart to give maximum seed production. In the greenhouse the F_1 seeds are planted in pots or in the soil of the greenhouse bench. Some artificial light may be necessary in the winter time in the northern climate, and frequently a complete fertilizer is needed.

If the pedigree method is used, the F_2 and succeeding generations, until bulked, should be grown in spaced planted progeny rows with 25 to 50 seeds per row. In certain studies, replication is desirable. The parental checks should be sown every 10 to 30 rows so that frequent comparisons can be made with them in selecting plants for further study.

Selections for disease resistance, height of plants, date heading, and any special characters such as head type, color of glumes, and type of awn (in the case of cereals) are made from plant rows in F_3 and later generations. In selecting for leaf rust, the plants must be marked several weeks before harvest. Selection for stem-rust resistance can be made at harvest time. The general vigor and habit of growth of the lines is observed in making selection and this observation used in conjunction with notes on specific characters. The individual plants harvested are threshed separately and the seed examined in the laboratory for type, size, shape, color, and plumpness. Seed of plants with inferior grain quality is discarded.

Disease tests usually are begun in the F_2 by subjecting the plants to an epidemic and selecting only resistant plants. In F_3 and later generations, the usual method is to plant separate nurseries in order to study reaction to the various diseases for which it is hoped to obtain resistant varieties. Resistant plants are then selected in these disease nurseries. In breeding for resistance to diseases in which the methods used in inducing the disease epidemic results in abnormal plant growth, the common practice is to grow special disease nurseries but to select plants or lines from a duplicate nursery grown under normal conditions, lines found to be susceptible in the disease nursery being discarded.

Quality tests are made whenever possible. In some breeding programs these can be begun in F_2. Usually these tests are made in later generations when a greater bulk of seed or plant material is available. This is true particularly when the cost of making the quality tests is great. The material is then purified, and all otherwise undesirable lines are discarded before being tested for quality.

Special characters may be studied by growing the F_3 and subsequent generations under environmental conditions that will bring out the differentiation desired.

During the segregating generations, it is desirable to grow rows of the parents and of the best available standard variety at frequent intervals throughout the nursery. Only plants and progeny rows that appear equal to the standards in all respects should be selected.

METHODS OF BREEDING

Several methods of breeding self-pollinated crops through the use of hybridization have proved satisfactory for particular problems. These may be classified as:

1. The pedigree method.
2. The bulk method.
3. The backcross method.
4. Multiple crosses.

In these and other problems of a similar nature, the larger the populations during the segregating generations the more chances there are of obtaining the combination of characters that are desired. The more complex the inheritance the greater the need for larger numbers. Although the exact combination of characters desired may not be recovered in F_2, there is still the possibility of obtaining it in F_3 or later segregating generations. When two factors are closely linked, their recombination will be obtained infrequently in F_2 but secured more easily in F_3 by growing the progeny of F_2 plants that contain one of the two desired characters. In most crosses it is a sensible plan to grow as large an F_2 population as can be sampled adequately in F_3.

Pedigree Method.—This method consists of (1) making a cross between two parents possessing the characters that it is desired to combine in a new variety, (2) growing the material in spaced plant rows so that individual plants may be studied, and (3) keeping a system of records so as to be able to trace individuals from one generation to the next. Numerous systems of keeping progeny records are available and will be chosen to suit the needs of the investigator. An outline of some of the methods was given in Chap. V.

The number of seeds sown, length of row or size of plot, and number of generations grown before bulking the plants in progeny rows will vary considerably with the different crops. For cereal crops the following procedure will illustrate the steps involved.

1. Grow sufficient F_1 plants to produce the desired amount of seed for F_2. Compare the F_1 plants with the parent varieties, note dominance of characters, and discard selfs. Seed from the identical parent plants used in producing each F_1 progeny may be

grown beside the F_1 and, in critical studies, seed from these parent plants grown for comparison with F_2 and later generations.

2. Grow 2000 to 10,000 individually spaced F_2 plants. In F_3 and subsequent generations, grow 1000 or more progeny rows each year from seed of individual plants selected the previous year. Select on the row basis first, and then select the best plants in these rows. Discard any lines found to be undesirable in disease nurseries.

3. Bulk the seed of rows when homozygous. This usually is done in F_4 to F_6. At Minnesota, with small grains, pedigree selection is continued until F_5, when promising apparently homozygous lines are bulked for the yield trials. Some lines may be continued in F_6 from selected F_5 plants before bulking. Lines not homozygous in F_6 are discarded unless very promising. Apparent homozygosity is determined by examination of the individual plants of a line, in the field, for observable agronomic or disease characters, and then the plants are harvested and threshed individually and the seed examined before bulking the seed for yield trials.

4. Conduct yield trials, and release for distribution to the growers as described in Chap. V.

Bulk Method.—This method consists of growing the material in a bulk plot, usually from the F_2 to about the F_6 generations, inclusive, followed by head selection in F_6. By the F_6 generations, a high proportion of the plants will be homozygous for most observable characters. The bulk plots can be subjected to disease epidemics and special conditions as an aid in selection. Natural selection probably will eliminate some of the weaker types. The progenies of plants selected in F_6 are tested in the manner described for improvement by selection in Chap. V.

Because of the ease with which crosses can be carried in bulk, a greater number can be grown in this way during the segregating generations than by the pedigree method. However, in the absence of selection through F_6 a higher proportion of the population will be undesirable than with the pedigree method, in which case careful selection over a period of years would have eliminated more of the undesirable types. As a consequence, it would be necessary to select more plants in F_6 for testing in plant rows than would be necessary to test in F_5 or F_6 with the pedigree method.

Harrington (1937) suggested a modification of the bulk method called the mass-pedigree method. This involves a combination of the two methods. The material is grown in bulk until a favorable season provides conditions for efficient selection. Then head selections are made and grown the following year in progeny rows as described for the pedigree method. The essential feature of this method is the growing of the crosses in bulk until a year favorable for efficient selection occurs, when single-plant selections are made and the pedigree method used from that time onward. In order to make selections in bulk plots, selection would need to be made on the head rather than plant basis.

Frequently a great deal can be learned regarding the genetics of the material during the segregating generations when the pedigree method is followed. Such is impossible with the bulk method.

Backcross Method.—This method is used primarily when it is desirable to transfer one or two simply inherited characters of the nonrecurrent parent to the recurrent parent, which is usually a highly improved variety of a desirable agronomic type.

An outline is given of the steps to be followed:

1. Grow the F_1 and backcross to the recurrent parent.
2. Grow 50 to 200 individual backcrossed plants in spaced progeny rows.
3. Select desirable individuals, *i.e.*, those containing the characters to be selected from the nonrecurrent parent.
4. Backcross these selected plants to the recurrent parent. Continue backcrossing and selecting from 2 to 6 generations. In some cases, it may be necessary to study the progenies of selected plants before making the next backcross.
5. After backcrossing is finished, the material is handled in the same manner as outlined for the segregating generations by the pedigree method. There is, however, this difference. After several generations of backcrossing, many of the factors from the recurrent parent will be homozygous, and fewer generations need be grown in individual plant rows before bulking. Only a few years of yield tests will be required also, since most of the lines will be similar to the recurrent parent in all but the one or two of the characters added from the nonrecurrent parent.

Multiple Crosses.—Harlan and Martini (1940) have suggested the use of compound crosses. The method may be illustrated by assuming that eight varieties are to be combined. A series of bridging crosses is made as follows: $a \times b$, $c \times d$, $e \times f$, $g \times h$. In a second mating, the F_1 plants will be crossed to produce the double crosses $(a \times b) \times (c \times d)$ and $(e \times f) \times (g \times h)$. In

the third mating the double crosses will be combined as follows: $[(a \times b) \times (c \times d)] \times [(e \times f) \times (g \times h)]$. As segregation will have taken place at the time the second cross is made, a greater number of crosses would need to be made than in the first mating. In the third cross, a very large number of seeds would be desired, since every seed contains essentially a different genotype and will result, presumably, in a new combination of characters. This procedure offers some promise of obtaining unusual combinations of factors, leading to the production of exceptional segregates. Its disadvantages would lie in the fact that several of the parent varieties probably would be undesirable for certain characters, and crosses between them would lead to the production of a higher proportion of plants with these undesirable traits. Large populations would need to be grown during the segregating generations following the compound crosses. These can be carried by either the pedigree or bulk method of breeding.

COMBINING ABILITY

Plant breeders observe very frequently that more desirable segregates are obtained from some crosses than from others. Some varieties are good parents, as judged by their ability to transmit high yield and quality to their progeny in crosses; others are less desirable. In the production of hybrid corn, the fact that some inbred lines transmit higher yielding ability to their F_1 crosses than do others, when crossed with a series of inbreds, has been known for many years. The classification of varieties of self-pollinated crops as to whether they will transmit high yield in crosses has hardly begun.

Harrington (1932) suggested that an analysis of the characters that could be studied in an F_2 population will provide a means of predicting the value of a cross. Harlan and Martini (1940) crossed 28 varieties of barley in all possible combinations of two each, making 378 crosses. These crosses were each carried in bulk plots, without selection, until the eighth generation and then space-planted. Plant selections were then made from each cross and tested in progeny rows the next year. Since each variety was crossed with each of the other 27, the potential value of each variety, in crosses, could be determined from the average yield of the selections made in crosses involving each parent in turn. The varieties Atlas, Club Mariot, Minia, Trebi, and Sandrel

produced an unusually high percentage of superior selections. Crosses involving Glabron produced very few. Some varieties that had not been sufficiently promising in nursery tests to be grown in plots were found to be superior parents.

Harrington (1940) and Immer (1941) studied the yield of bulk crosses in wheat and barley, respectively, in early generations as a means of determining the comparative breeding value of different crosses. The study by Immer will be reviewed briefly.

Six barley crosses were compared with one another in F_1, F_2, F_3, and F_4 and with the parents. The yields of F_1 and parental checks were determined from rows of 11 plants per cross or variety, spaced 5 in. apart, and replicated six times. The tests in F_2, F_3, and F_4 were made in five replicated rod-row plots, the parents being included. The seed for F_3 and F_4 tests was a random sample from F_2 and F_3, respectively.

In Table 4 is given the average yield of each pair of parents for 3 years, expressed in percentage of the mean yield of parents for all crosses. The yields in F_1 to F_4 are expressed in percentage of the average mean yield of the parents in the six crosses for the year or years in which the test was made.

TABLE 4.—YIELD OF PARENT VARIETIES AND F_1, F_2, F_3, F_4 CROSSES IN BARLEY, EXPRESSED IN PERCENTAGE OF THE AVERAGE YIELD OF THE PARENTS GROWN THE SAME YEAR AS THE CROSSES

Cross	1938		1939–1940					1940
	Average of parents	F_1	Parent varieties ♀	Parent varieties ♂	Parent varieties Average	F_2	F_3	F_4
Barbless × Chevron	121	151	127	93	110	119	114	120
Barbless × Minsturdi	118	186	127	74	101	125	114	83
Velvet × Chevron	117	142	111	93	102	140	118	111
Barbless × Olli	87	116	127	109	118	137	124	117
Barbless × C.I. 2492	80	81	127	51	89	115	105	100
Velvet × C.I. 2492	76	89	111	51	81	111	101	99
Average	100	128	...	..	100	125	113	105

The crosses of Barbless × Olli and Velvet × Chevron produced the highest yields in F_2 and F_3 and were among the highest in F_4. They were intermediate in yield in F_1. The two crosses

involving C.I. 2492 were relatively low in yield in all four generations tested.

It appears that tests of bulk crosses in F_2 or F_3 may be used as a means of discarding entire crosses in the early segregating generations, and the plant breeder then can make selections only from the crosses that promise the greatest proportion of high-yielding segregates. Testing in several different localities and for more than 1 year would be advisable.

From the study by Immer, it appeared that the F_1 could not be used to determine satisfactorily the potential value of a group of crosses. Since the amount of seed in F_1 is very much limited, space planting must be resorted to. It was found that some of the varieties and crosses responded in a differential manner when space-planted 5 in. apart as compared with seeding in rod rows at the regular rate for such trials.

As information on the sources of good germ plasm in crop plants accumulates, as measured by combining ability in crosses, the outstanding parent varieties will be isolated and used more extensively in breeding programs.

In using the pedigree or bulk methods of breeding, the first yield test is obtained usually in F_6 to F_8. It would be highly desirable to know the yielding capacity of different strains from a cross and to discard the low-yielding ones before F_6 to F_8. Although no experimental data are available, it would appear that replicated yield trials in F_4, from bulked seed of F_3 lines that themselves were each the progeny of an individual F_2 plant, should supply information on the relative yielding capacity of such strains. A space-planted nursery of the same strains could be grown the same year and single-plant selections made. In F_5 plant-progeny rows would be grown from the strains found to produce high yields in the yield trials. The plant-progeny rows could be bulked in F_5 or F_6, if homozygous, for regular yield trials of pure material. By this procedure, a higher proportion of the strains in the regular yield trials would be expected to give satisfactory yields.

CHAPTER VII

THE BACKCROSS METHOD OF PLANT BREEDING

Backcrossing, when possible, is the most satisfactory method to use in genetic studies to determine linkage relations. It is also useful as an aid in developing a factorial hypothesis. Harlan and Pope (1922) pointed out its probable value in small-grain breeding and stated that it has been "largely if not entirely neglected in any definite breeding programs to produce progeny of specific types." They suggested the probability that there were many instances in which backcrossing would be of greater value than the more common method of selecting during the segregating generations after making suitable crosses. Before giving some of the results obtained, it seems desirable to discuss the principles involved.

GENETIC EXPECTATIONS FROM BACKCROSSING

As used in plant breeding, backcrossing seems to be a logical procedure when it is advantageous to add one or two characters, each of which is conditioned by one or two genetic factors, to an otherwise desirable variety. The general plan of study may be outlined as follows:

1. Selection of parents for crossing.
   A variety, A, with desirable characters but lacking one or two characters that are dependent upon only a few genetic factors.
   A variety, B, containing these one or two characters that A lacks.
2. Backcrossing of the F_1 of $A \times B$ to A; selection for the one or two desirable characters of B, if they are dominant, in each backcross generation and again backcrossing of these selected plants to A.
   Repetition of the process as seems necessary. In this illustration, A and B are called the recurrent and nonrecurrent parent, respectively. Recessive characters of the nonrecurrent parent can be carried along by growing sufficient plants in each backcross generation and by making sufficient backcrosses to be sure some plants are heterozygous for the recessive factors that it is desired to add to the recurrent parent.
3. Selection in the selfed progeny from plants carrying the factors obtained from B until homozygosis for the characters of the B parent is reached.

In self-pollinated plants, the new lines obtained may be compared with each other and with parent A in field trials and the strain of greatest promise increased and distributed as an improved variety if it performs satisfactorily. In cross-pollinated plants it seems necessary to produce several desirable lines and recombine these to produce a synthetic variety or to use certain of these lines to produce F_1 crosses for the utilization of hybrid vigor. With a crop like corn, hybrid seed may be produced by this method. With asexually propagated crops, the more desirable crosses may be propagated by asexual methods.

Richey (1927) has given the percentages of plants homozygous for the n factors entering the cross only from the recurring homozygous parent in each of r successive generations, calculated from the formula $\left(\frac{2^r - 1}{2^r}\right)^n$. These percentages are given in Table 5.

TABLE 5.—PERCENTAGES OF PLANTS HOMOZYGOUS FOR THE n FACTORS ENTERING A CROSS ONLY FROM THE HOMOZYGOUS RECURRENT PARENT TO WHICH THE CROSS AND THE RESULTING PROGENIES ARE MATED IN EACH OF r SUCCESSIVE GENERATIONS*

Number of factor pairs, n	Number of generations of back pollinating, r									
	1	2	3	4	5	6	7	8	9	10
1†	50	75	88	94	97	98	99	100−	100−	100−
5	3	24	51	72	85	92	96	98	99	100−
10		6	26	52	73	85	92	96	98	99
15	.	1	13	38	62	79	89	94	97	99
20	.	.	7	28	53	73	85	92	96	98
30	..		2	14	39	62	79	89	94	97
40	..	..	..	8	28	53	73	86	92	96
50	..	..	.	4	20	46	68	82	91	95
75	.		.	..	9	31	56	75	86	93
100	..	.	..		4	21	46	68	82	91

* Subject to errors incident to the use of 6-place logarithms.

† The values for $n = 1$ give also the percentages of homozygous factor pairs in the entire population, regardless of the value of n.

This formula is the same as that used for finding the percentage of homozygous individuals in different segregating generations after a cross. In the segregating generations after a cross, only one-half of the homozygous individuals are of the desired genotype. For example, the F_2 generation of the cross between AA and aa will consist of $1AA + 2Aa + 1aa$. One-half of the

progeny are homozygous, but of these only one-half are of the AA genotype. If, instead of selfing the F_1, Aa, it is backcrossed to AA, $1AA$ to $1Aa$ will be obtained. In this case, one-half of the total progeny are of the desired genotype AA.

Richey gave a summary also of the number of plants required in F_2 and the first backcross generation to obtain a single individual with the required genotype when one to eight factor pairs were involved. These results, which are calculated on the basis of independent inheritance, are given in Table 6.

TABLE 6.—PROGENY REQUIRED TO HAVE ONE DOMINANT HOMOZYGOUS INDIVIDUAL

Method	Number of factor pairs							
	1	2	3	4	5	6	7	8
F_1, selfed	4	16	64	256	1024	4096	16,384	65,536
F_1, backcrossed to homozygous dominant .	2	4	8	16	32	64	128	256

With a difference of five factor pairs in the parents, for example, the calculated expectation in F_2 is only 1 individual out of every 1024, with all 5 factor pairs in a dominant homozygous condition, whereas for the first backcross generation the theoretical expectation is 1 out of every 32 that contain all 5 factor pairs in a dominant homozygous condition.

Linkage may be involved between a factor C for one of the desirable characters of the recurring parent A and the recessive condition of the dominant factor R carried in parent B that it is hoped to add to A. Suppose, in addition, that there may be 10 other factor pairs involved in which the A parent carries the desired genotype.

Parent A carries CC linked with rr and 10 other dominant factors, and parent B carries cc linked with RR, the latter being the character desired to add to parent A. It may be supposed that C and r show 10 per cent recombination. Selection for R in the backcross generations will tend to make it difficult to obtain the desirable factor C, but as C is brought in from the recurring parent in each backcross the chances of a crossover and the desired

combination of *CCRr* seems better in backcrosses than by the pedigree plan. The reason for this is that the *Rr* genotypes are selected each year and the *C* factor brought in from the recurring parent. After a crossover takes place, then linkage of *C* and *R* will tend to make these combinations more frequent than under a system of independent inheritance of these 2 factor pairs. The 10 remaining dominant factors will be recovered according to the usual theoretical expectation.

The value of the backcross method may be better appreciated by giving a few illustrations.

CANTALOUPES RESISTANT TO POWDERY MILDEW, *ERYSIPHE CICHORACEARUM*

This disease, according to Jones (1932), cannot be controlled satisfactorily by spraying or dusting. A mixed lot of seed from India produced numerous plants practically free from disease, but the melons were of very low quality. These conditions gave an ideal setup for a backcross plan of breeding, for resistance appeared to be a simple dominant over susceptibility, and desirable melons lacking resistance to powdery mildew were available.

The method used consisted of crossing the resistance Hindu melon with commercial cantaloupes, selecting for resistance in the backcross generations, and recrossing these selected resistant plants to commercial cantaloupes. After cantaloupes of desired quality were obtained, selection was followed until strains were obtained that were homozygous for resistance. Seed from several strains was combined to give the necessary vigor of growth in the new variety. This material should be as uniform for other characters as the original variety.

BREEDING BUNT-RESISTANT WHEATS

Briggs (1930) described a project that was started in 1922 for adding the bunt resistance of Martin to important commercial varieties of wheat grown in California, Martin being selected for one parent because it had proved completely resistant to bunt on the Pacific coast and because there appeared to be only one factor pair involved in this type of resistance. Subsequent discussions of the backcross method by Briggs (1935, 1938) emphasize the extensive use of the method by Briggs and coworkers.

The plan outlined originally by Briggs is as follows:

1922. Martin (resistant variety) × Baart (commercial, susceptible variety)
1923. F_1 × Baart
1924. Plants segregated 1 resistant: 1 susceptible
1925. Plants segregated 3 resistant: 1 susceptible
1926. Progeny of resistant plants segregated giving 1 homozygous: 2 heterozygous rows
Homozygous resistant plants were crossed with Baart
1927. F_1 × Baart

It will be noted that the progeny of the first backcross segregated in a ratio of 1 resistant: 1 susceptible, showing resistance to be dominant. The progeny of the resistant plants of the backcross segregated in a ratio of 3 resistant: 1 susceptible, and the progeny of these resistant plants gave 1 homozygous resistant line: 2 heterozygous, on the average.

The resistant line was then used for the second backcross. The reason for studying the progeny of selfed lines after the first backcross, until homozygosis is again obtained, is to eliminate the possibility of selecting a plant for backcrossing that did not carry factors for resistance, since some plants escaped infection even though genotypically susceptible.

The practical accomplishments of Briggs,[1] in California, demonstrate the value of the backcross method. In 1922, a program was started to incorporate the bunt resistance of Martin in all commercial wheats grown in California, including the varieties Baart and White Federation. In 1930, a program was initiated to add the stem-rust resistance obtained from Hope to Baart and White Federation in addition to bunt resistance. By crossing the bunt-resistant Baart and White Federation with rust-resistant strains of the two varieties, resistances to both diseases were combined. From these studies, 11 bunt-resistant varieties, two of these being resistant also to stem rust, have been produced. The first group of varieties obtained from the program have been grown extensively. The improved varieties are practically identical with the original varieties except for the character of bunt resistance.

A program has been adopted by Briggs and his coworkers of compositing 70 or more backcross lines for each variety. These

[1] Unpublished information kindly furnished by Fred N. Briggs.

lines retain the name of their original commercial type, with the year of increase appended to designate them from their susceptible counterpart. It has been found that the mean yield of all lines is almost exactly the same as that of the original parent when bunt is not a factor in yielding ability.

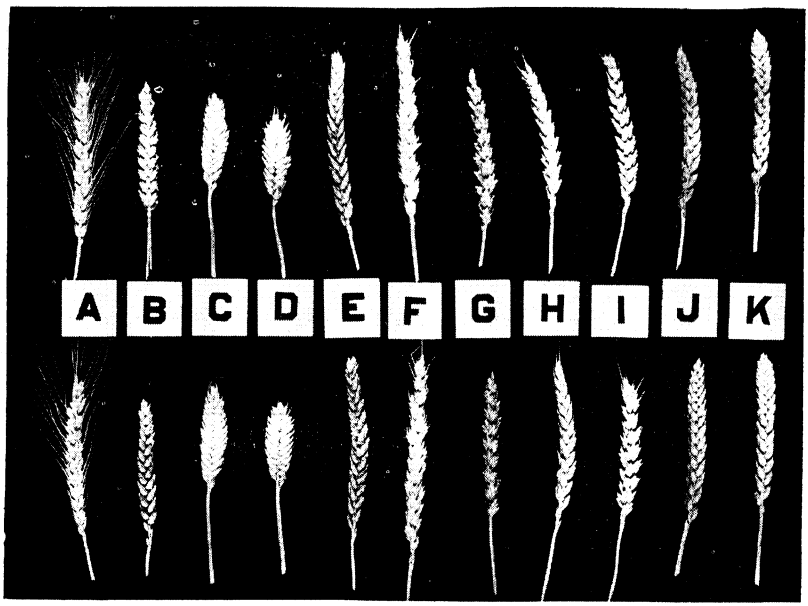


FIG. 13.—Top row: (*A*) Baart 38; (*B*) White Federation 38; (*C*) Big Club 37; (*D*) Poso 41; (*E*) Sonora 37; (*F*) Pacific Bluestem 37; (*G*) Ramona 41; (*H*) Bunyip 41; (*I*) Escondido 41; (*J*) Federation 41; and (*K*) Onas 41, all carrying the Martin factor for resistance to bunt. *Tilletia tritici* and the first two, Baart 38 and White Federation 38, also have resistance to stem rust, *Puccinia graminis tritici*, from Hope. The corresponding heads in the bottom row are from the original susceptible parents.

BREEDING RUST-RESISTANT SNAPDRAGONS

Emsweller and Jones (1934) have described the development of varieties of the cultivated snapdragon resistant to rust, *Puccinia antirrhini* D. & H., and emphasized the extent to which the disease has become of commercial importance. From the progeny of seed obtained originally from E. B. Mains, of Indiana, several plants were found that under favorable conditions for infection proved entirely free from rust. Resistance was found to be dominant in crosses and resistance and susceptibility to be conditioned by a single main factor pair, although minor modifying factors that influenced the extent of resistance were present also.

These writers describe their experiments in which they used the backcross method to combine this resistance with the flower color and plant habit of standard commercial varieties and state that progress was very encouraging.

STUDIES AT THE MINNESOTA STATION

For several years, backcross studies have been carried on at Minnesota, where the problems involved appeared to be of such a nature that the backcross method seems the most logical plan of breeding. Several of these problems will be outlined briefly to give further illustrations of the principles involved.

Disease Resistance in Wheat.—In spring and winter wheat, desirable commercial varieties are available that are of good agronomic habit and of high milling and baking quality. These varieties lack one or two characters of outstanding importance, notably the high resistance of Hope and H44 wheats to stem and leaf rusts. Since resistance to both diseases is relatively simple in inheritance, the addition of these types of resistance to desirable varieties available can be most logically and easily accomplished by the backcross method of breeding.

Its use may be illustrated by the improvement of Thatcher through crossing with Hope, primarily to add leaf-rust resistance from Hope and increase stem-rust resistance. Thatcher is the standard for yielding ability, desirable agronomic characters, and milling and baking value. It is not as stem-rust resistant as Hope and is very susceptible to leaf rust. The following information was furnished by E. R. Ausemus, of the Division of Cereal Crops and Diseases, U. S. Department of Agriculture, who is stationed at University Farm, St. Paul, Minnesota, and who has charge of the wheat-breeding program.

HISTORY OF BACKCROSS (THATCHER × HOPE) × THATCHER

Year	Plan	Place
1930	Original cross	Field
1930–1931	First backcross	Greenhouse
1931	Second backcross	Field
1932–1937	Pedigree selection	Field

Leaf-rust epidemics were obtained only in 1932 and 1935. Stem-rust epidemics were obtained each year.

Yield in bushels per acre, test weight, and leaf-rust reaction, from trials made in 1938, when there was a severe rust epidemic in the northwest spring-wheat area, are given in Table 7.

TABLE 7.—YIELD IN BUSHELS PER ACRE AND TEST WEIGHT IN REPLICATED ROD-ROW TRIALS AT UNIVERSITY FARM, 1938. LEAF-RUST REACTION IN ROD ROWS (AGRONOMY) AND IN THE RUST NURSERY (R.N.)

Variety	Yield, bu.	Test weight	Rust, per cent			
			Leaf		Stem	
			Agron.	R.N.	Agron.	R.N.
Thatcher	18	47	70	80	T	3
B.C., II-31-2	31	55	2	T	T	T
B.C., II-31-6	30	54	T	5	T	T
B.C., II-31-14	32	54	3	T	T	T

Illustrations with Corn.—The backcross method seems well adapted to use with cross-pollinated crops that are being bred by controlled pollination and selection. In one instance, two inbred lines of Crosby sweet corn have been selected that combine well together to give a vigorous F_1 cross. In common with many strains of Crosby sweet corn, these lines have the undesirable characteristic of toughness of pericarp. Two lines of Golden Bantam have been selected that excel in flavor and tenderness of pericarp. One of these has been crossed with one of the strains of Crosby and the other Golden Bantam line with the second strain of Crosby.

The F_1 generation is intermediate in tenderness and can be differentiated from the tough pericarp parent by puncture tests when the canning stage is reached. This can be accomplished by stripping back the husk at this stage and puncturing several kernels in the middle of the ear and recording the pressure required. The backcross method has been used in this problem.

It is not known how many genes are concerned in tenderness of pericarp. Inbred lines are available that give considerable ranges in mean values when measured by the puncture test. The results in the following summary show that by selection it is possible to differentiate heterozygous ears, by means of puncture tests, from the homozygous tough parent. Data given in Table 8 are summarized from the studies of Johnson and Hayes (1938).

The major factor or factors for tenderness was retained in the heterozygous condition by selection in each of the succeeding generations of backcrossing. Plants of the first backcross generation, (I × H)I, were pollinated by pollen from the I inbred parent, tested for puncture-test values, and those that were intermediate for tenderness were selected as parents for the next backcross generation. After three generations of backcrossing, selection in self-pollinated lines was used to isolate homozygous tender pericarp inbreds that resembled the recurrent tough pericarp parent in most other characters.

TABLE 8.—FREQUENCY DISTRIBUTION OF PUNCTURE-TEST VALUES OF EARS FROM INDIVIDUAL PLANTS OF THE PROGENY OF BACKCROSSES TO THE TOUGH PERICARP PARENT WITH SELECTION FOR TENDERNESS

Culture	Year	Puncture-test values									
		240	260	280	300	320	340	360	380	400	420
I	1935	..	..	..	..	..	..	11	40	37	4
I	1936	..	..	..	..	..	..	11	46	43	
I	1937	..	..	..	.	..	2	16	8		
H	1936	14	28	33	6	1					
H	1937	1	19	16	10	2					
(I × H)I	1935	..	.	4	12	23	23	15	4	1	
(I × H)I_2	1936		..	5	14	36	40	26	13	2	
(I × H)I_3	1937		..	2	28	43	65	40	15	6	

Recent studies of inheritance of smut reaction in corn show that the character is relatively complex from the genetic standpoint. Attempts have been made to gain some idea of the number of factors involved in crosses of resistant inbred lines with highly susceptible lines that have known interchanges for certain chromosomes. The interchange plants are semisterile, and the point of interchange may be handled in the same manner as a dominant factor. To determine what parts of the chromosome map are involved in relation to factors for resistance and susceptibility to smut, studies were made of smut reaction in relation to points of interchange. Studies at Minnesota of crosses of susceptible lines carrying interchanges with two different selfed lines resistant to smut, one from Minn. 13, and the other from

Rustler, were carried on. The F_1 plants were crossed to the resistant parents and in backcross and F_2 generations the X^2 test for independence was used to determine associations between smut reaction and points of interchange. In each series of crosses, at least three different chromosomal regions carried inherited factors for reaction to smut, and the regions from the Rustler crosses were entirely different than those for the Minn. 13 crosses. Similar results have been reported by Burnham and Cartledge (1939).

Various workers have found it relatively easy, however, to select inbred lines resistant to smut from crosses between resistant and susceptible inbreds and from selection in self-pollinated lines from commercial varieties. Resistance seems to be relative and probably functions under normal conditions against all physiologic races of smut. The inbred line B164 is used as a male parent in producing Minhybrid 301 and also in Pioneer 355, two three-way crosses adapted to southern Minnesota. As grown in Minnesota, B164 is highly susceptible to smut, and under normal conditions as high as 90 per cent of the plants may be infected. New lines, resembling B164, have been obtained from a cross between B164 and culture 37, a resistant line of Minn. 23. Two backcrosses to B164, followed by 3 years of self-pollination, isolated several inbred lines that had only 10 per cent of smut when grown adjacent to B164 that showed 85 to 90 per cent smut.

Another illustration may be given with a corn problem now being investigated. Seed from one of the double crosses grown commercially in Minnesota, known as Minhybrid 401, is of mixed color, carrying both yellow and white kernels on the same ear. It was obtained by crossing the F_1 cross of two inbred lines of yellow endosperm corn obtained from Minn. 13, lines 11 and 14, with two white endosperm inbred lines of Rustler, 15 and 19.

It was desired to change the color of the white endosperm lines of Rustler from white to yellow without changing their combining ability. These Rustler lines carry the dominant whitecap factor *Wc*, which, in the presence of yellow endosperm, causes whitecap. The yellow lines lack this factor but contain a dominant factor for yellow endosperm color *Y*. The expected results for several backcrosses will be given on the basis of independent inheritance of *Wcwc* and *Yy*.

Unrelated yellow endosperm lines were selected to cross with these white endosperm lines, the problem being to obtain yellow

endosperm lines that in other characters resemble the white lines of Rustler used in the double crosses. During the backcrossing period, selected plants in each backcross generation were crossed with particular Rustler inbreds that were used as the recurrent parent. In each backcross generation, the gametes of the non-recurrent parent will be given, and the percentage of seeds heterozygous for *Wc* and *Y* will be given also.

In each backcross generation, whitecap, yellow-endosperm kernels were selected. Some of these will be homozygous for whitecap and will be of no value. Others will be heterozygous for both the whitecap and yellow-endosperm factors. These are the combinations desired, and the proportions of such combinations are given for each backcross generation.

1. Parent genotypes, *WcWcyy* and *wcwcYY*.
   F_1 genotype *WcwcYy*.
   F_1 gametes *WcY*, *wcY*, *Wcy*, *wcy*.
2. First backcross genotypes and phenotypes.
   a. 1 *WcWcYy* whitecap, yellow endosperm.
   1 *WcwcYy* whitecap, yellow endosperm.
   1 *WcWcyy* white endosperm.
   1 *Wcwcyy* white endosperm.
   b. Per cent heterozygous for *Y*, 50.
   Per cent heterozygous for *Wc*, 50.
   Per cent heterozygous for *Wc* and *Y*, 25.
   c. Select to backcross to the Rustler inbred line.
   Genotypes: *WcWcYy*, *WcwcYy*.
   Gametes: 3 *WcY*, 1 *wcY*, 3 *Wcy*, 1 *wcy*.
3. Second backcross genotypes and phenotypes.
   a. 3 *WcWcYy* whitecap, yellow endosperm.
   1 *WcwcYy* whitecap, yellow endosperm.
   3 *WcWcyy* white endosperm.
   1 *Wcwcyy* white endosperm.
   b. Per cent heterozygous for *Y*, 50.
   Per cent heterozygous for *Wc*, 25.
   Per cent heterozygous for *Y* and *Wc*, 12.5.
   c. Select to backcross to the Rustler inbred line.
   Genotypes: 3 *WcWcYy*, 1 *WcwcYy*.
   Gametes: 7 *WcY*, 1 *wcY*, 7 *Wcy*, 1 *wcy*.
4. Third backcross genotypes and phenotypes.
   a. 7 *WcWcYy* whitecap, yellow endosperm.
   1 *WcwcYy* whitecap, yellow endosperm.
   7 *WcWcyy* white endosperm.
   1 *Wcwcyy* white endosperm.
   b. Per cent heterozygous for *Y*, 50.
   Per cent heterozygous for *Wc*, 12.5.
   Per cent heterozygous for *Y* and *Wc*, 6.25.

In most problems of this kind, three backcrosses will probably be all that are necessary. At the end of this period, in the absence of linkage, seven-eighths of the genotype of the Rustler inbred line will be recovered, according to theory, and if the whitecap, yellow-endosperm seeds are selected, one out of every eight will be heterozygous for both *Wc* and *Y*. When planted and self-pollinated, segregation will occur for both *Wc* and *Y*. From selfed ears, yellow seeds that do not carry the dominant whitecap factor should be planted the following year, and of these one plant out of every three, on the average, when self-pollinated, will breed true for yellow color. In backcross studies with inbred lines of corn, it is generally agreed that after three backcrosses the progeny very closely resemble the recurrent parent in general habit of growth.

From the breeding results with corn, some general conclusions may be emphasized. When it is desired to add certain definite characters to an otherwise desirable inbred, selection may be practiced for these characters during the generations of backcrossing when the character is a dominant one. Backcrossing is followed by selection in self-pollinated lines. If the character is recessive, selection may be made after each backcross by self-pollination and selection for the homozygous recessive before making further backcrosses, or selection may be made for the recessive character obtained from the nonrecurrent parent during the segregating generations when selfing is practiced.

In certain corn crosses, one of the selfed parents excelled in most easily observed characters, such as resistance to smut, good root system, and ability to withstand lodging and in general vigor. The other parent was less desirable in most easily observed characters. The F_1 crosses were vigorous and far superior to either of their inbred parents. It seemed relatively easy by backcrossing and selection to obtain inbred lines with marked improvement over the more undesirable parent but rather difficult to obtain lines better than the more desirable inbred from backcrosses to this inbred as the recurrent parent. A backcross program may be most advantageously pursued when definite characters may be selected for. The problem seems more difficult in selecting for such characters as vigor of growth that are dependent upon the interaction of many factors.

CHAPTER VIII

BREEDING FOR DISEASE AND INSECT RESISTANCE

The principles underlying the breeding for resistance to disease or insect attacks are much the same as for other characters. There is, however, one important difference. In breeding for disease and insect resistance, one is dealing with two series of heritable factors: (1) the heritable characters of the host plant and (2) the heritable differences in the organism.

In most cases, the plant breeder is interested primarily in the reaction of selected strains, varieties, and hybrids under conditions to which they will be exposed normally when grown for economic purposes. The breeding program should be carried on under conditions as similar to those to be encountered by the commercial varieties as is feasible.

It is in breeding for resistance to diseases that the modern plant breeder has made some of his greatest contributions. The development of special methods for producing artificially induced epiphytotics and fundamental studies of the genetics of the host and parasite have laid the foundations for the planned breeding programs of the present. This fundamental information, although coming slowly in the beginning, has increased rapidly in recent years, leading to a scientific appreciation of the problems and, in many instances, to a sound basis for solution.

THE IMPORTANCE OF DISEASE RESISTANCE

The development and utilization of disease-resistant varieties is one of the important methods of disease control. When the required resistance can be obtained in combination with other necessary qualities, it seems fair to conclude that the growing of disease-resistant varieties is the most desirable method of controlling diseases. Much has been accomplished already, but there is an almost unlimited opportunity for further work. Seasonal variations in climatic factors are the major causes of seasonal variations in the yielding ability of crops, and to a considerable extent these variations cannot be controlled. The

development of disease-resistant varieties will help to stabilize yields, since disease epidemics are among the more important causes of wide fluctuations in the yield of crops from season to season.

With disease resistance, as with other characters, it is important to use as large numbers as possible in the breeding program, although the source of material is of greater importance. A good illustration of these two points is the present plan for breeding high-quality European grapes that are resistant to *Phylloxera*, the vine louse, and *Peronospora*, the vine mildew. According to Baur (1931) the cost of attempting to control these pests amounts to between 30 and 50 million marks annually. American varieties of grapes, *Vitis rupestris*, are resistant to both *Phylloxera* and *Peronospora*. The American grapes are of low quality, whereas the European varieties, *V. vitifera*, are of high quality but susceptible. Baur stated that crosses between these two species are fertile, and he had under way large-scale experiments to select, from the segregates of hybrids between the two species, varieties that excel in both quality and resistance. The tests for mildew resistance are made at the plant-breeding station at Müncheberg, and those for resistance to vine louse at the Institute for Phylloxera Research at Naumberg. In another publication (Baur 1933), the statement has been made that from 5 to 7 million F_2 seedlings are grown yearly. They are tested for reaction to mildew, and only the seedlings resistant to mildew are saved and tested for yield and quality. Those that survive are then tested for resistance to the vine louse.

Flax is grown as a cash crop in the United States in the spring-wheat region of the central northwest, largely for the oil content in the seed. In earlier years, it was grown chiefly on new breaking, *i.e.*, prairie soil not previously under cultivation. Eventually no new soil was available, and it became necessary to grow the crop on old land. Serious losses from wilt occurred, and it seems very probable that flax could not have continued to be a successful crop without the development of resistant varieties.

In 1901, Bolley grew normal varieties on "wilt-sick soil" and isolated the organism *Fusarium lini* responsible for the disease. He observed (1901) the fact that some plants were not seriously injured even under epidemic conditions. These and other studies continued until the present time have emphasized the value of

wilt resistance. Today no improved varieties of flax are introduced unless they have the necessary resistance to wilt.

In more recent times, wilt-resistant varieties have been developed for many plants. In 1915, cabbage growers in Wisconsin were so discouraged because of the ravages of cabbage yellows (*F. conglutinans*) that they were about to abandon cabbage growing. Jones and Gilman (1915) observed a few plants in fields infected with the disease that apparently escaped the disease. These were selected and proved to be resistant by progeny trial. Today cabbage yellows is no longer serious. Wilt-resistant strains of all important varieties grown in Wisconsin have been developed.

By similar methods, wilt-resistant varieties of tomatoes have been obtained. Edgerton (1918) and others, in Louisiana, have developed an improved technic for selection. Seed was planted in sterilized soil and then inoculated with a pure culture of the wilt-producing organism *F. lycopersici*. Those seedlings that were injured by wilt were pulled up and discarded and the resistant seedlings transplanted to a field that was known to contain infected soil. Lines breeding true for resistance were tested for other characters and distributed when satisfactory.

Orton (1913) developed watermelons resistant to wilt, caused by *F. niveum*, by crossing an inedible, resistant citron with a good-quality, susceptible watermelon and selecting for resistance and quality.

About 1920, farmers in western New York were greatly concerned over the bean-mosaic disease. Emerson and coworkers had learned that a variety known as M. A. C. Robust, developed by selection in Michigan, was resistant to mosaic. They introduced the new variety, planting single rows in the center of the farmer's fields, and were greatly pleased with the great resistance that this variety exhibited. To quote from Emerson, "I have no hesitation in saying that resistance to this disease, mosaic, saved the pea-bean industry in Western New York."

Some of the accomplishments during the last 25 years in the production of disease-resistant varieties of vegetables have been summarized by Rieman (1939). A study of lists of varieties of vegetables offered for sale by the seed trade was made in 1914. At that time, less than a dozen resistant varieties were listed by two of the leading American vegetable seed houses, and most

of these proved of doubtful value. In 1939, over 80 resistant varieties were listed, and 20 or more were recognized by the seed trade as leading varieties. These 80 disease-resistant varieties included 2 varieties of asparagus resistant to rust; 3 varieties of snap-bean resistant to mosaic and 2 resistant to bean rust; 10 varieties of cabbage resistant to cabbage yellows; 1 variety of celery resistant to *Fusarium* wilt; 6 varieties of sweet corn resistant to Stewart's bacterial wilt and 2 resistant to the corn-ear worm; 9 varieties of lettuce resistant to brown blight, 3 resistant to downy mildew, and 2 resistant to tip burn; 2 varieties of cantaloupe resistant to powdery mildew; 29 varieties of peas resistant to *Fusarium* wilt; 2 varieties of spinach resistant to mosaic; and 7 varieties of tomatoes resistant to *Fusarium* wilt.

Rieman stated that the method of control through breeding disease-resistant varieties involved four important steps: (1) the recognition of disease symptoms and the identification of the causal organism, (2) the isolation of fertile, resistant breeding stocks, (3) the development of true-breeding disease-resistant varieties through crossing and selection, and (4) the production and distribution of pure high-quality seeds of the resistant varieties in commercial quantities.

METHODS OF BREEDING FOR DISEASE AND INSECT RESISTANCE

The methods of breeding will be discussed under several headings, namely: (1) the search for resistant materials, (2) the artificial production of the disease epidemic, (3) the plan of breeding, and (4) a study of fundamental problems that aid in a logical attack on the breeding problem.

The Search for Resistant Material

The search for resistant varieties or strains is a logical first step. It is highly desirable, in beginning an attack on a disease problem, to make a collection of local and foreign varieties and to test their reaction under epidemic conditions. If reaction to the disease has been studied by investigators in other states or other countries, varieties found by them to be resistant would, of course, be tested first to determine their reaction under the environmental conditions of the new investigation.

The methods of breeding varieties with the necessary resistance do not differ from those used for other characters. In some

cases, hybrids between susceptible varieties may give resistant plants in the segregating generations. If disease resistance is an important problem, at least one of the parents of a hybrid should have the desired resistance whenever such resistant varieties are available.

With disease resistance, as with other characters, it is extremely important to learn as much as possible regarding the genetic factors responsible for resistance. It must be appreciated that the organism causing the disease frequently comprises several (or many) strains or physiologic races, which in some cases can be differentiated only by their manner of reaction to a series of varieties known as differential hosts. Almost 200 such physiologic races have been found in *Puccinia graminis tritici*.

For stem rust of oats (*P. graminis avenae*), there is a complete correlation between the manner of reaction of seedlings and the reaction of the mature plants to the same physiologic races (Smith 1934). In barley, Brookins (1940) found the reaction of mature plants to a large collection of physiologic races in the field to be controlled by the same genetic factor pair controlling reaction to races 19, 36, and 56 in the seedling stage. Resistance to stem rust in wheat may be placed in two main classes: (1) physiological, where the reaction to a specific race of the pathogen is relatively the same throughout the life of the plant, and (2) mature-plant resistance, in which case the genes concerned produce resistant mature plants independently of their reaction in the seedling stage.

It is necessary in most cases to make a survey of the physiologic races normally prevalent in the locality and to breed for resistance to all prevalent forms. This is essential in dealing with physiological resistance, since a variety may be very resistant to one physiologic race and highly susceptible to another. With mature-plant resistance to stem rust of wheat and smut in corn, varieties tend to react in the same manner to all physiologic races of the pathogen. In studies of disease resistance, it is very helpful to have the definite cooperation of the plant pathologist, who will lead in the studies of the organism and in methods of creating artificial epidemics. After these problems have been solved, the breeding of disease-resistant varieties is not greatly different from that of breeding for any other character.

Artificial Production of Epiphytotics

It is not within the scope of this book to describe methods of inducing disease or insect epidemics for all crops in which breeding for resistance is being undertaken. A brief description will be given for some of the major diseases of the common field crops grown in the northern United States and Canada. These will be described for the crop and disease, or insect pest, involved.

Black-stem rust of wheat, oats, and barley (Puccinia graminis).

1. Field.
   a. Plant susceptible varieties as borders around the outside of the plot and through the alleys.
   b. Obtain as many physiologic races as possible that have been found in the region. Increase these races on seedlings of susceptible varieties in the greenhouse, and use a mixture of races for inoculations in the field.
   c. Transfer rusted plants from the greenhouse to the border rows in the field.
   d. Hypodermically inoculate the border rows with a mixture of uredospores of all the races increased in the greenhouse. A mixture of about 30 races has been used at Minnesota in recent years.
   e. Spray the plants with an aqueous spore suspension late in the evening, when there is probability of dew, or just before or after a rain.
   f. Keep the soil moist in a dry season to delay premature ripening and prolong the length of the susceptible period.
2. Greenhouse.
   When there is correlation between seedling reaction in the greenhouse and reaction in the field from heading to maturity, it is of advantage to study the progeny of selected plants in the greenhouse as an aid in discarding susceptible material.
   a. Grow from 15 to 20 seedlings from selected plants in small pots until first leaves are well developed.
   b. Spray seedlings with water, and inoculate by brushing with leaves of infected seedlings, or apply the spores with a scalpel.
   c. Place the pots in an incubation chamber under high humidity for 48 hr. The chamber may have a glass top to admit light.
   d. Transfer pots to greenhouse bench, and observe the reaction when rust has developed to the point where maximum differentiation is obtained.

Leaf rust of wheat (Puccinia triticina) and crown rust of oats (P. coronata).

1. Increase rust of the races to be used on seedlings in the greenhouse.
2. Plant susceptible varieties as border rows around and through the field-rust nursery.

3. Spray the plots with an aqueous suspension of all races usually prevalent in the locality. The plants should be inoculated on a still night, when the humidity is high. Seedlings may be inoculated in the field when about 8 in. high.
4. Irrigate, if necessary, to maintain susceptibility over a period of time.
5. Tag resistant plants, if the lines are segregating. Final selections are made at harvest time from these resistant plants.

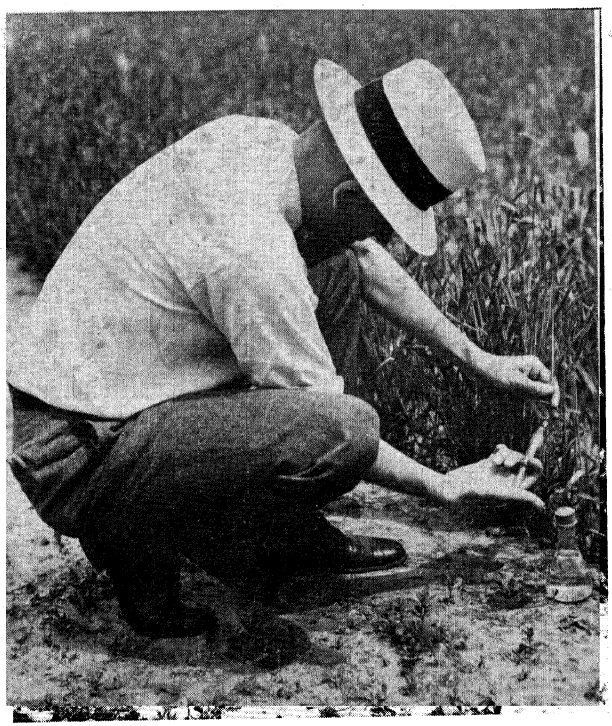


FIG. 14.—Hypodermic inoculation of border rows of susceptible plants with a spore suspension of a collection of races of stem rust.

Bunt of wheat (*Tilletia tritici*).

1. Obtain as many collections of smut as possible from a wide area in order to obtain a large number of races. Do not use collections of smut from too wide an area or from foreign countries, for there is danger in introducing more virulent forms of the pathogen.
2. Dust the seed of varieties or hybrids to be tested with a mixture of spores from all the collections, using about 1 g. of smut per 100 cc. of grain.
3. Plant the seed as early as possible, if spring wheat, or when the soil is sufficiently cool and relatively dry. The optimum temperature for infection is approximately 12°C.

Loose smut of oats (Ustilago avenae), covered smut of oats (U. levis), covered smut of barley (U. hordei), and intermediate smut of barley (U. medians).

1. Obtain as many collections as possible in the area in which the variety may be grown ultimately.
2. Make an aqueous suspension of the spores at the rate of ½ g. of spores to 100 cc. of water.
3. Submerge seed in about one and one-half to two times its volume of the spore suspension.
4. Subject to sufficiently high vacuum to withdraw air from under the hulls. Two evacuations in succession are preferable.
5. Pour off the suspension, and dry the seed.
6. The seed may be stored for several weeks before planting without greatly affecting the efficiency of inoculation.
7. Plant when temperature is moderately high and the soil relatively dry.

Flax rust (Melampsora lini).

1. Increase the rust in the greenhouse.
2. Plant border rows of susceptible varieties around and through the field nursery.
3. Spray a water suspension of spores on border rows of susceptible varieties. When rust appears, make spore suspensions, and spray the rust over the entire nursery, or brush the plots with infected plants from the border rows.
4. Save bundles of rusted flax plants in the fall, and scatter the straw on the plots in the spring, when the plants are coming up.
5. Grow the flax in a place where the temperature is relatively low and humidity is high, since these conditions are conducive to the development of an epidemic.

Flax wilt (Fusarium lini).

1. In the greenhouse.
   a. Plant susceptible varieties to be tested in sterile soil inoculated with the cultures of the causal organism.
2. In the field.
   a. Collect soil from fields where flax wilt has been prevalent, and mix this with the soil in the test plot.
   b. Grow a mixture of races of the causal organism on sterile grain, nutrient agar, or in liquid media, and inoculate the soil.
   c. Plant the varieties and strains of flax to be tested in this "wilt nursery."
   d. Use the same plot every year.

Fusarial head blight (scab) of wheat and barley.

1. Increase the different organisms on sterile oats or wheat in jars or flasks in the laboratory.
2. At time of heading, cover the field-test plot with a cloth tent.

3. After heading, spray the plants every day or two with a spore suspension made from the different organisms. Continue the spraying until the grain is in the soft-dough stage, or until a satisfactory epidemic has been produced.
4. Spray the plants, soil, and tent with water to maintain high humidity in the tent.

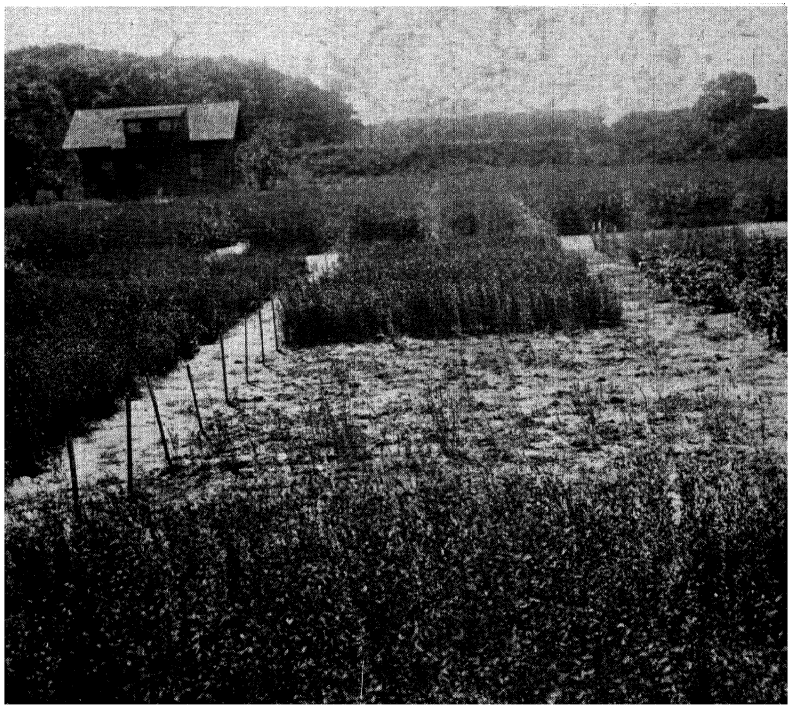


FIG. 15.—Flax wilt nursery showing resistant and susceptible strains, the latter entirely killed by wilt.

Corn smut (*Ustilago zeae*).

1. Make as many collections of smut as possible in the region in which the corn may be grown.
2. Mix the collections of chlamydospores with manure, and spread between the rows when the corn is 6 to 12 in. high.
3. Dust chlamydospores on the plants, or spray with a spore suspension, three or four times during the growing period of the corn.
4. Use the same field as a "smut nursery" every year.

Loose smut of sorghum (*Sphacelotheca cruenta*) *and covered smut of sorghum* (*S. sorghi*).

1. Make collections the previous year from as many sources as possible.
2. Dust the spores on the seed before planting.

3. Plant the seed when fairly high temperatures are assured. The optimum temperature for infection is about 27°C., and very little infection will result below 15°C.

Head smut of sorghum and corn (Sorosporium reilianum).

1. Make as many collections of the organism as possible.
2. Mix chlamydospores with soil, and spread this over the field-test plot, or apply with seed at planting time.
3. Plant in relatively dry soil.
4. Use the same plot every year.

Hessian-fly injury of wheat.

1. Plant replicated Hessian-fly nurseries in short rows, with check rows of varieties of known reaction at regular intervals.
2. Import infested stubble from the localities in which the wheat is to be grown ultimately. "Flaxseed," secured by dissecting plants from preceding tests, may be used also. Place these between the rows, and sprinkle with water.
3. Several biologic or physiologic races of the fly are known. The races produce a different reaction on different varieties of wheat.
4. In the greenhouse, grow the plants in pots, and transfer to an enclosure in the insectary after the plants are well tillered. Allow adult flies to lay eggs, and after emergence of larvae, return the pots to the regular greenhouse. After the fly has reached the "flaxseed" stage, the plants are dissected and the infestation recorded.

METHODS OF BREEDING

It is desirable that the early generations of selections and the segregating generations from crosses be grown under controlled epidemic conditions, so that the resistant strains can be selected and the others eliminated. It is generally considered that a mixture of races, or collections, of the pathogen, from the general region in which the improved crop will be grown, should be used in producing the epidemic. There is, however, some danger in carrying inoculum from one locality to another, since a virulent form of the disease may be introduced. For this reason, the more promising of the selections may be grown in several localities and exposed to an epidemic in each locality, created by using disease organisms collected in that locality.

The fact that plant pathogens are composed of physiologic races that can be differentiated only by their manner of reaction on a series of varieties known as differential hosts is appreciated today by students of plant breeding. A knowledge of the number and nature of these physiologic races is important. Two

general methods may be followed. These are not necessarily in opposition to each other but may go hand in hand. One consists of a study and isolation of as many physiologic races of the organism as are available and a determination of the reaction of parents and hybrids to individual races. The other method consists of using a collection of races in producing the epidemic and selecting only those strains that are resistant to all races.

Knowing the reaction of parental varieties to special races makes possible a definite breeding program in relation to these races. Thus, with the physiologic races of stem rust of wheat, *Puccinia graminis tritici*, to which Kanred is immune both in the seedling and in the mature-plant stage, immunity from a rather large group of races is dependent upon a single genetic factor. Breeding for resistance to a single one of these races gives an accurate idea of the reaction to all races from which Kanred is immune.

An illustration may be given from the studies of Smith (1934) with respect to stem rust of oats.

TABLE 9.—REACTION OF VARIETIES OF OATS TO NINE RACES OF STEM RUST

Variety	Reaction to races							
	1	2	3	4	5	6	7	8
White Russian	*R**	*R*	*S*†	*S*	*R*	*S*	*S*	*R*
Rainbow	*R*	*R*	*R*	*S*	*R*	*S*	*R*	*S*
Joanette	*R*	*S*	*R*	*R*	*S*	*S*	*S*	*S*
Victory	*S*	*S*	*S*	*S*	*S*	*S*	*S*	*S*

* *R* = resistant.
† *S* = susceptible.

Races 1, 2, and 5 are the most common in the central northwest section of the United States. It seems probable that reaction to races 1, 2, 3, 5, and 7 may be dependent upon an allelic series of three factors, one governing resistance to all five races, another resistance to races 1, 2, and 5 and susceptibility to 3 and 7, and still another susceptibility to all five races. Rainbow, a selection from Green Russian, made at the North Dakota Agricultural Experiment Station, is resistant to all five races; White Russian is resistant to 1, 2, and 5 but susceptible to 3 and 7. The resistance of Rainbow is somewhat greater to races 1, 2, and 5 than that of

White Russian, and as it is resistant also to races 3 and 7, its type of resistance is more desirable than that of White Russian.

It is advantageous in some cases to duplicate the nursery, making two planting dates. The material that gives the most satisfactory disease epidemic may be used for selection and the material from the other planting date discarded.

Breeding for disease resistance can be carried on most advantageously by carrying on the studies of disease reaction as a part of the main breeding project, selecting for disease reaction, for quality, and for agronomic characters at the same time, although in some cases in special nurseries. In this way, if selection must be made for several characters, progenies that excel in all these characters may be used as a basis of selection.

STUDY OF FUNDAMENTAL PROBLEMS

The importance of a knowledge of the pathogen and the environmental conditions favorable for the development of the disease will be appreciated by students with training in plant pathology. From extensive studies with many organisms, more especially species of rusts, smuts, powdery mildews, pasmo, *Fusarium* root rots, *Helminthosporium*, *Colletotrichum*, and others, it has been learned that many and perhaps most of the organisms causing diseases are composed of numerous physiologic races that can be differentiated only by the manner of their reaction on a series of varieties known as differential hosts.

The identification of physiologic races of pathogens responsible for particular diseases has made possible a more definite attack on fundamental problems, such as the effect of environmental conditions in causing marked changes in disease reaction, the ability of different races to develop to epidemic proportions as affected by environment, the screening effect of varieties as a means of modifying the proportion of particular races present during any particular season, and other similar problems. These questions belong logically in the subject-matter field of plant pathology and cannot be handled adequately here. An illustration of the manner of identification of physiologic races based on host reaction will be given for loose smut of oats, *Ustilago levis*, after Reed (1940). Differential species and varieties used include *Avena brevis; A. strigosa; A. sativa*, varieties Black Diamond, Black Mesdag, Black Norway, Danish Island, and

Monarch; *A. sativa orientales*, variety Green Mountain; *A. nuda*, variety Hull-less; *A. byzantina*, variety Fulghum. The manner of differentiation is given in Table 10, taken from Reed.

TABLE 10.—DIFFERENTIATION OF SPECIALIZED RACES OF *Ustilago levis* (K. & S.) Magn.

	Race
a. Monarch *susceptible*	
b. Black Mesdag *susceptible*	
c. Fulghum *susceptible*	
d. Black Norway *susceptible*	6
d. Black Norway *resistant*	
e. Black Diamond *susceptible*	7
e. Black Diamond *resistant*	8
c. Fulghum *resistant*	9
b. Black Mesdag *resistant*	
c. Black Norway *susceptible*	
d. Green Mountain *susceptible*	13
d. Green Mountain *resistant*	14
c. Black Norway *resistant*	
d. Green Mountain *susceptible*	
e. Danish Island *susceptible*	
f. Hull-less *susceptible*	11
f. Hull-less *resistant*	12
e. Danish Island *resistant*	
f. Avena strigosa susceptible	1
f. Avena strigosa resistant	10
d. Green Mountain *resistant*	
e. Black Diamond *susceptible*	4
e. Black Diamond *resistant*	3
a. Monarch *resistant*	
b. Avena brevis susceptible	2
b. Avena brevis resistant	
c. Hull-less *susceptible*	5

A summary of the status of physiologic races has been made by Reed (1935) and Stakman *et al.* (1935). Studies of physiologic races and the use of races common to the region where the improved resistant varieties, when obtained, will be grown, will aid the breeder in producing varieties with the necessary resistance to races common in the locality. A knowledge of stability of pathogenicity and the basis of new races in terms of mutation or hybridization is essential.

The genetics of plant pathogens has been reviewed recently by Stakman *et al.* (1940). A few examples will be cited to illustrate principles involved.

Selfing of individual physiologic races of black-stem rust on the barberry often leads to the isolation of several to many different races. Hybridization between races within the same species or between different species of rusts may lead to the production of new races. When two races of stem rust, homozygous for pathogenicity, are crossed, the F_1 resembles one or the other parent, and there appears to be Mendelian dominance. If heterozygous races are crossed, new races with greater virulence than either parent may be obtained.

A knowledge of the nature and frequency of mutation for pathogenicity is fundamental, especially the probability of a change from a mild form of the disease to a more virulent form. From studies made so far, it would appear that mutations that lead to an increase in virulence are relatively infrequent.

A knowledge of the genetics of the pathogen is very important in planning breeding studies in which the breeding for disease resistance is one of the major objectives. Close cooperation between plant breeders and plant pathologists would appear to be essential if maximum progress were to be made.

A knowledge of the nature and causes of disease resistance in terms of physiology, morphology, or functional behavior are basic to a real understanding of the problem.

Walker (1935) discusses the nature of resistance to cabbage yellows and describes two categories of inherited resistance that have been obtained. In Type *A*, resistance is dominant to susceptibility and controlled by a single dominant gene. All collections of the parasite react in the same manner to plants of Type *A*, and the behavior is constant over a wide range of temperatures. There is a second type of resistance, known as Type *B*, which is complex in inheritance, and the reaction varies with the temperature, all plants being susceptible at a soil temperature of from 22 to 24°C. Thus, plants of the Type *A* resistant group may be selected by raising the soil temperatures to 24°C. While the physiological or morphological differences that differentiate the two types of resistance are unknown, the information regarding genetic differences makes it possible to breed for the required resistance.

From the plant-breeding standpoint, there are two major types of resistance to stem rust. Stakman (1914) showed that resistance to certain physiologic races of *Puccinia graminis tritici* is

due to physiological incompatability between the host plant and the fungus. The germ tube of the fungus may enter resistant varieties, but the fungus is unable to establish itself to the extent that it can cause severe injury. Physiological or protoplasmic resistance functions throughout the life of the plant. This appears to be the type of resistance to stem rust found in oats (Smith 1934) and barley (Brookins 1940).

The second general type of resistance to stem rust in wheat is called mature-plant resistance. Some varieties are susceptible to one or more races in the seedling stage yet resistant to these and other races in the stage from heading to maturity. The exact nature of mature-plant resistance is not known. Whatever its nature, the mode of inheritance of this type of resistance appears to be relatively simple in crosses differentiated by this type of resistance and involving crosses with Hope or H44. It appears to function against all physiologic races found in the spring-wheat region of the United States and Canada.

A knowledge of the mode of inheritance of reaction to disease helps in planning the breeding program. The genetics of reaction to disease frequently can be studied during the segregating generations of hybrids made during the course of the regular breeding program at little additional cost. Such information, analyzed and reported, will leave an ever-increasing store of information that will be available as a guide to future investigations.

The extent of correlation between reaction to disease and other important character differences should be determined. A knowledge of genetic linkage may serve as a guide in planning breeding programs or in fundamental inheritance studies.

Cooperation between breeders in different states and countries will be of benefit to all. New facts will become known earlier, and free exchange of material and ideas will speed up the solution of all breeding problems. Jealousies leading to the withholding of information from others working on similar problems will impede such progress. The testing of material in many places leads to a more rapid determination of its real value.

Painter *et al.* (1940) described extensive experiments in Kansas in breeding wheat resistant to Hessian fly [*Phytophaga destructor* (Say)]. From crosses of Marquillo, a Hessian-fly resistant spring wheat, with desirable varieties of winter wheat susceptible to Hessian-fly attacks, these workers have produced strains of winter

wheat that combined resistance to Hessian fly and tolerance to wheat jointworm, with resistance to leaf rust, stem rust, bunt, and mildew. The fly resistance of Marquillo is probably derived from its Iumillo durum parent. In crosses, the resistance of Marquillo tends to be recessive and due to more than a single genetic factor. Resistance appears to be due to the interaction of three separate heritable mechanisms: low larval survival, ability to withstand infestation, and, under some conditions, low oviposition. The best explanation of the differences in varietal behavior in different regions lies in the presence of biological strains of the fly, which differ in their ability to infest different varieties of wheat.

CHAPTER IX

INHERITANCE IN WHEAT

The relationship of *Triticum* and related genera was given in Chap. II by means of genom analysis. In vulgare wheats, for example, there are three genoms, called *A*, *B*, and *C*, each containing a set of seven chromosomes. Thus, for many characters, there may be three pairs of factors for the dominant and recessive condition. If, as some believe, the basic chromosome number of the *Gramineae* is five instead of seven, a greater number of duplicate factors than three could be accounted for, depending upon the means by which the basic number five was changed to seven. Instances of three duplicate factors are common in members of the Spelta group, including *Triticum vulgare*, *T. compactum*, and *T. spelta*, and two duplicate factors have been found in members of the Emmer group, including *T. dicoccum*, *T. durum*, *T. turgidum*, and *T. polonicum*. It is well to recall the fact that *T. monococcum* carries genom *A*; *T. dicoccum*, *T. durum*, *T. turgidum*, and *T. polonicum*, genoms *A* and *B*; and *T. vulgare*, *T. spelta*, and *T. compactum*, genoms *A*, *B*, and *C*.

Studies of glume shape help to illustrate types of inheritance that may be expected in amphidiploids.

Glume Shape.—Extensive studies of glume shape and keel development have been made by Watkins (1940), who has summarized the present status of the problem. Wheat species may be described as follows:

Hexaploids.

vulgare—round, loose glumes and tough rachis.
speltoid—keeled, thick glumes and tough rachis.
spelta—keeled, very thick glumes and brittle rachis.

Tetraploids.

durum, *turgidum*—keeled, loose glumes and tough rachis.
dicoccum—keeled, thick glumes and brittle rachis.

Watkins concludes that the tetraploids contain two sets of factors, perhaps representing completely linked groups of genes with the genetic formulas

dicoccum K^dK^d K^dK^d
turgidum KK KK

He also suggests the formulas for the hexaploids to be

vulgare kk KK K^dK^d
speltoid KK KK K^dK^d
spelta K^sK^s KK K^dK^d

In crosses between *turgidum* and *dicoccum*, the F_1 has the formula K^dK K^dK and is somewhat intermediate, resembling *dicoccum* more closely than *turgidum*, with rather thick glumes and intermediate brittle rachis. In the F_1 meiosis, autosyndesis probably occurs as first suggested by Darlington (1927) to explain the reason for a lack of complete recovery in the segregating generations of parental glume lengths in crosses between *polonicum* × *durum*, called shift by Engledow (1920). In the cross of *turgidum* × *dicoccum*, the F_1 gametes presumably are all K^dK because of pairing in F_1 in the form of K^dK^dKK, leading to a true breeding form in F_2 that resembles the F_1.

In crosses of *vulgare* with *dicoccum* and *turgidum*, respectively, it was concluded that K^d remained unpaired when crossed to *turgidum* and K, when crossed to *dicoccum*, which is in agreement with the lack of pairing of K and K^d in the *turgidum* × *dicoccum* cross.

With this hypothesis, k, K, K^d, and K^s are allelic groups of completely linked genes, and K^d and K^s are similar or identical in effect. The glume, keel, and rachis characters that differentiate the five wheat species are caused by variations in a single chromosome complement, present four times in tetraploids and six in hexaploids.

Watkins presents evidence also of a linkage between the factor pair for bearded vs. tip awns, called B_1b_1, and for glume condition, K^dk or Kk, with a recombination value of approximately 41 per cent.

Awnedness.—There are three major groups of wheats: awnless, awnleted, and bearded. The awnleted groups produce short awns, these being longer and more numerous, usually near the

tip of the spike in one group of wheats, and more evenly distributed in another group. Probably as a result of minor modifying factors or allelic series, there are intermediate classes also that in some cases breed true. Homozygous awnleted varieties may differ in the extent of development of awns, and definite classification of the genotype of homozygous material is difficult without a breeding test.

Watkins and Ellerton (1940) have postulated the following factors for different types of awns in hexaploid wheats:

B_1, the gene for tipped 1 belonging to the allelic series B_1, b_1, and $b_1{}^a$. A few awn tips up to 1 to 2 cm. in length are produced, the longest tips being found near the top of the spike.

b_1, recessive gene for bearded.

$b_1{}^a$, belonging to a series of allels including B_1 and b_1; producing half-awned types with short awns.

B_2, the gene for tipped 2 belonging to the allelic series B_2, b_2 and perhaps containing A. Awns reduced to a few short tips occurring from top to bottom of the spike.

b_2, recessive gene for bearded in the presence of the homozygous condition for b_1.

A, another gene for the half-awned condition that may be an allele of the b_2 series and that gives half-awned types in the presence of the recessive condition for b_1 and b_2.

Hd, a gene that reduces the length of the awns, making them curved and twisted near the base.

Possible combinations of factors include $b_1b_1\ b_2b_2\ hdhd$, bearded; $B_1B_1\ b_2b_2\ hdhd$, tipped 1; $b_1b_1\ B_2B_2\ hdhd$, tipped 2; $b_1b_1\ b_2b_2\ HdHd$, hooded; and $B_1B_1\ B_2B_2\ hdhd$, beardless; and $b_1b_1\ B_2B_2\ HdHd$, hooded beardless.

B_1 is linked with genes for pubescent node, square-headedness, and keeled glumes.

The Howards (1915), in India, explained the results of a cross between awnless and bearded varieties on the basis of two pairs of factors, the homozygous dominant condition of both leading to the production of the fully bearded condition. Quisenberry and Clark (1933) crossed two awnleted wheats, Quality and Sonora, and obtained true breeding awned, awnleted, and awnless wheats, respectively, in F_3 in addition to a wide range of segregating groups. They used the same hypothesis as given originally by the Howards except that they considered awnless to be the dominant group rather than bearded.

The student should not be confused by the question of dominance. It is relatively easy to differentiate the F_1 from either the awnleted or fully bearded parents when representative varieties are crossed that differ in awnedness. Percival (1921) reported F_2 segregations approximating a 1:2:1 ratio, with the intermediate or heterozygous condition producing longer tipped awns that often extended down the head to a greater extent than in the awnleted parent.

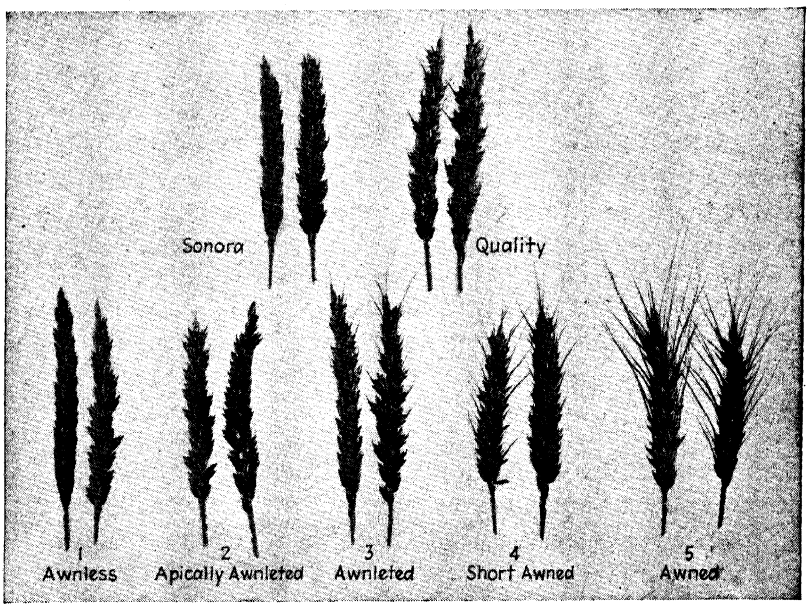


FIG. 16.—Quality and Sonora, two types of tipped awn wheats, and five different types of progeny obtained in F_2. Two new homozygous types were selected, awnless and bearded. (*After Quisenberry and Clark*, 1933.)

Chaff Characters.—Color of glumes is a varietal character ranging from deep brownish red color to colorless. Segregation in crosses between colored vs. colorless with 3:1 and 15:1 ratios has been reported (Biffin 1905, Kezer and Boyack 1918). There are inherited differences in color of awns that have been reported to segregate in simple ratios (Howards, 1915).

Hairy chaff is a varietal character used in classification. In crosses between members of the Emmer group with varieties of *Triticum vulgare*, some cases of complete association have been reported between chaff color and the hairy chaff character

(Biffin 1905, Engledow 1914, Henkemeyer 1915, Kezer and Boyack 1918). The Howards have reported two kinds of hairs on the glumes of Rivet wheat. In a cross between two Indian varieties that differed in the sorts of hairs produced on the chaff, a ratio of 15 pubescent: 1 smooth was obtained in F_2. It seems probable that the two pairs of factors for pubescent vs. smooth chaff are carried in separate genoms and therefore are independently inherited. Since there are at least two pairs of factors for pubescence, this would explain variations in linkage relations between chaff colors and pubescence.

Seed Characters.—Color of seed, resulting from a brownish red pigment in the testa, has been commonly used in varietal classification and in determining market grades. It is a plant character and is not immediately affected by cross-pollination. Red is dominant over white, and from one to three pairs of duplicate factors are involved, as first shown by Nilsson Ehle (1911*a*) and later found by many other workers. Segregating ratios 3:1, 15:1, and 63:1 have been observed, and crosses between two varieties, both breeding true for red seed color, may give plants lacking red seed color in F_2, provided that the parents differ in the genetic factors involved. One parent, for example, may be $R_1R_1\ r_2r_2$, whereas the other may have the genotype $r_1r_1\ R_2R_2$, leading to the production of some white-seeded plants of the genetic constitution $r_1r_1\ r_2r_2$.

Texture of seed is used also in varietal classification and in market grades. Biffin (1916) observed the immediate effect of cross-pollination in a cross of Rivet, a corneous seeded variety belonging to *Triticum turgidum*, with a soft Polish variety of *T. polonicum*. In crosses of corneous-seeded durums with the soft-seeded variety Sonora, belonging to *T. vulgare*, Freeman (1918) observed variation in texture of seed in F_1 with hard, intermediate, and soft-seeded kernels on the same plant. Hard seeds of the F_1 tended to give more hard-seeded plants in F_2 than the progeny of soft seeds from F_1 plants. Freeman carried the study through F_4. He explained his results on the basis of two pairs of factors, the heterozygous condition being intermediate in soft-starch production. Since the endosperm results from the union of two polar nuclei with a male generative cell, there could be a range from zero to six factors for soft starch. The type of soft starch worked with by Freeman is different from the

type called yellow berry, which is conditioned by inheritance but is easily modified by environmental conditions.

Spike Density.—Crosses between *Triticum compactum* with *T. vulgare* by Spillman (1909) and Gaines (1917) have shown one main factor for compactness of head. In similar crosses, Parker (1914) concluded that multiple factors were involved. Nilsson-Ehle (1911*a*) studied crosses of Swedish Club (compact) with Squarehead, a mid-dense-headed type, obtaining compact heads in F_1 and segregation into compact, mid-dense, and lax in F_2. He explained his results by the hypothesis of C, a factor for compactness epistatic to L_1 and L_2, factors for length of internode carried by Swedish Club and the recessive condition carried by Squarehead, $cc\ l_1l_1\ l_2l_2$. F_2 plants of the phenotype $c\ L_1L_2$ were lax-headed. It is common, in crosses of vulgare with durum, to obtain Emmer-like wheats with very dense heads. Stewart's (1926) results from a cross of Sevier with Federation, two varieties of *T. vulgare* show that transgressive segregation for head density may occur. Sevier is somewhat more dense than Federation. The nature of segregation in F_2 was determined from F_3 progeny trials of F_2 plants selected at random. Homozygous dense, heterozygous, and homozygous lax forms occurred in a 1:2:1 ratio, although the dense forms were more dense than Sevier and the lax forms more lax than Federation.

Spring vs. Winter Habit.—The main character that differentiates spring from winter habit is heading behavior when wheat is sown in the spring. In the spring-wheat areas of the United States and Canada, winter wheat, when sown in the spring, remains in the rosette stage and fails to head. Spring wheat may be sown in the fall, and varieties of spring wheat often are fall-sown in those sections where the winters are mild. Spring-wheat varieties, as a rule, are less winter hardy than true winter wheats.

In crosses between spring and winter wheat, the spring habit, as a general rule, is completely dominant in F_1, and segregation occurs in F_2. The type of F_2 ratio obtained without doubt depends to a considerable extent upon the environmental conditions used to differentiate spring from winter habit. Ratios reported include simple ratios of spring to winter of 3:1 by Cooper (1923) and 15:1 by Nilsson-Leissner (1925); Vavilov and Kouznetsov (1921) and Aamodt (1923) obtained much more complex

ratios. In the F_2 of Kanred × Marquis, Aamodt classified plants from spring seeding for date of heading at weekly intervals into eight weekly periods and into a winter group consisting of types that failed to head. From 5253 F_2 plants, 980 headed as early as the spring parent, and 442 were classified as winter. The numbers of plants in other weekly periods for heading date were, respectively, from early to late, 1503, 883, 568, 417, 313, 128, and 19. Plants heading in F_2 as early as Marquis bred true for spring habit. Intermediates for date of heading bred true also in some cases. Studies by Hayes and Aamodt (1927) of cold resistance in crosses between Marquis with Minturki and Minhardi winter wheats included a study of growth habit also. A late heading type, when spring-sown, was selected that, when sown as winter wheat, was rather highly winter-hardy. When recrossed with Marquis and studied for cold resistance and for date of heading, when spring-sown, there appeared to be almost complete correlation between cold resistance and late heading (unpublished). In general, there is a close correlation between winter habit and cold resistance, but some wheats of winter habit are lacking in high cold resistance. Some varieties of spring wheats have considerably more resistance to winter killing when fall-sown than other varieties.

Powers (1934) studied spring vs. winter habit of growth in a cross of Hybrid 128 × Velvet Node. Under conditions at Pullman, Washington, the parents and hybrids were classified for date of ripening into weekly groups. The results were explained by the interaction of three main factor pairs, where *AA*, *BB*, and *cc* were factors for spring habit of growth and their alleles for winter habit. *AA* was epistatic to *bb* and *CC*, *BB* to *aa* and *CC*, and *cc* to *aa* and *bb*.

Stem-rust Reaction.—There are 177 physiologic races of *Puccinia graminis tritici* that have been differentiated by their mode of reaction on 12 host varieties when inoculated with stem rust in the greenhouse in the seedling stages [Stakman *et al.* (1935), Johnson and Newton (1940), and Dickson (1939)]. It is generally accepted that a variety of wheat, when resistant in the seedling stage to a particular physiologic race of the disease organism, usually is resistant under field conditions from heading to maturity to the same physiologic race. A variety of wheat, however, may be highly resistant to one physiologic

race and completely susceptible to another. New physiologic races originate (Craigie 1940) from hybridization on the barberry, the alternate host of black-stem rust, and in the presence of barberry bushes there is always the possibility of new physiologic races being developed.

The literature on the mode of inheritance of seedling reaction is very extensive. Illustrations will be given of several types of segregation. In a cross of H44-24 × Marquis (Goulden, Neatby, and Welsh 1928), where H44 was resistant to physiologic race 36 and Marquis susceptible, results were explained by supposing H44 to carry two duplicate factors for resistance, either alone in the homozygous dominant condition leading to semiresistance. Thus, the parental genotype of H44 was R_1R_1 R_2R_2, and of Marquis r_1r_1 r_2r_2. F_2 genotypes were as expected from a dihybrid ratio with the genotypes R_1R_1 R_2R_2, R_1R_1 R_2r_2, R_1r_1 R_2R_2, and R_1r_1 R_2r_2, showing the resistant type of phenotypic behavior, r_1r_1 R_2R_2, R_1R_1 r_2r_2, r_1r_1 R_2r_2 and R_1r_1 r_2r_2 being phenotypically semiresistant, whereas the double recessive r_1r_1 r_2r_2 was highly susceptible.

Harrington and Aamodt (1923) studied crosses between two durum wheats; Pentad, resistant in the seedling stages to physiologic race 34 and susceptible to race 1, and Mindum, which reacts in a reciprocal way to these two races. A single main genetic factor difference was responsible for reaction to each, and the two factors were independently inherited.

In a cross of Kanred, immune to over 11 physiologic races, with Marquis, which was susceptible to these same races (Aamodt 1923), immunity was dominant over resistance, and the manner of reaction to all races to which Kanred was immune and Marquis was susceptible was conditioned by a single genetic factor pair.

The few studies of seedling resistance that have been reviewed briefly are representative of the many extensive studies of the manner of inheritance of seedling reactions. The problem of obtaining in a single variety resistance in the seedling stages to all available races, and those races that may be found after further study, has seemed rather difficult, since new physiologic races are being found almost constantly, and the total number of races is increasing rapidly from year to year. Recently, however, the problem has appeared to be somewhat less difficult by the discovery of several new wheats, notably wheats from the

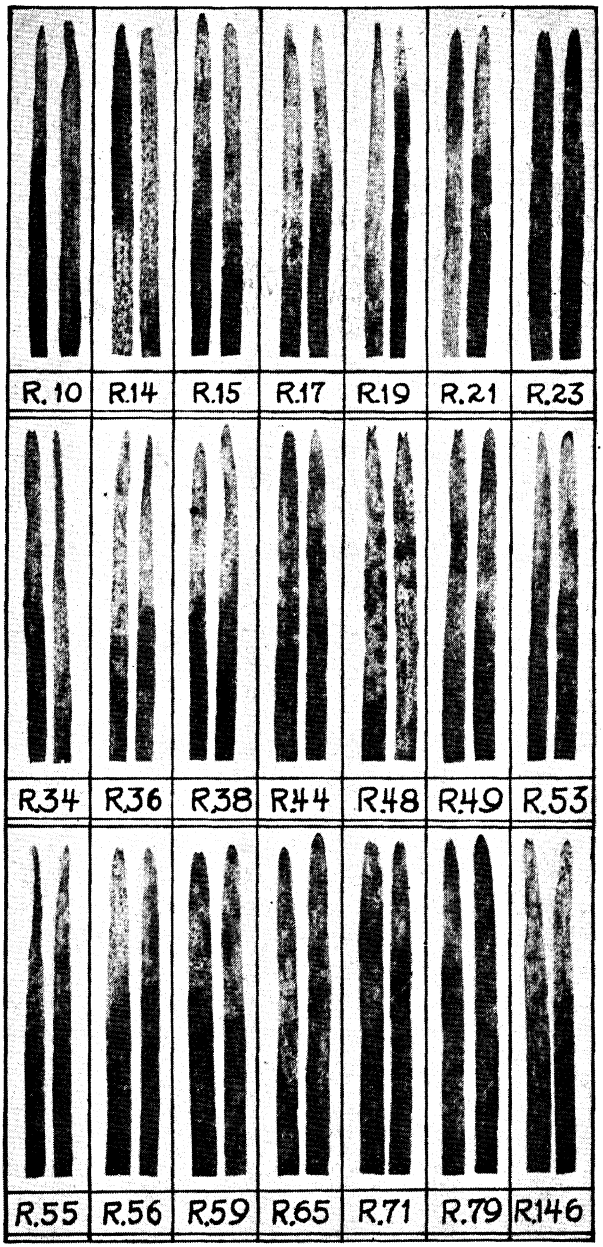


FIG. 17.—Reaction in the seedling stages of a selection from the cross, Kenya × Gular. Resistance to many physiologic races, both in the seedling and mature plant stages, is due to a single dominant factor. (*Courtesy of S. L. Macindoe.*)

Kenya Colony in Africa that have been described by Macindoe (1931) as resistant to the prevalent races in Australia. Some of these wheats have proved resistant in the seedling stages to 20 representative physiologic races (Peterson, Johnson, and Newton 1940) and have remained resistant also under field conditions, where 30 prevalent Canadian races have been used to produce the field epidemic.

The breeding of stem-rust-resistant vulgare types of wheat originally consisted of attempts to transfer stem-rust resistance from members of the Emmer wheat group to vulgare wheats by crosses between the 14 and 21 chromosome species. Hayes, Parker, and Kurtzweil (1920) obtained a vulgare type of wheat resistant to stem rust, later named Marquillo, from a cross of Iumillo, a durum variety, with Marquis. Marquillo proved less resistant than Iumillo, although under field conditions it has continued to be moderately resistant to a collection of prevalent physiologic races. A sister selection of Marquillo was crossed with a spring-wheat selection obtained from Kanred × Marquis that carried the Kanred type of immunity to several races. From this latter cross, Hayes, Stakman, and Aamodt (1925) concluded that resistance in the stages from heading to maturity to a collection of prevalent races was conditioned by two complementary factors and that susceptibility was dominant to resistance. It was found also that Marquillo and Thatcher, the latter selected from the cross (Marquis × Iumillo) × (Marquis × Kanred), were highly susceptible in the seedling stages to several of the prevalent races used in producing the field epidemic. The resistance of the Marquillo type, conditioned by two main factors in the field, proved independent in inheritance of the Kanred near immunity to certain physiologic races.

A more satisfactory type of stem-rust resistance was obtained by McFadden (1930) from crosses of a variety of *Triticum dicoccum*, Yaraslov Emmer, with Marquis. Two varieties obtained from this cross, Hope and H44, although not entirely satisfactory in agronomic characters, have in recent years been used by practically all breeders as a source of stem-rust resistance. These two wheats, like Thatcher and Marquillo, are susceptible in the seedling stages to several physiologic races that occur naturally both in United States and Canada, but both Hope and H44 have proved highly resistant in the mature-plant stages in

the field, from heading to maturity, to natural and artificial epidemics of black-stem rust. As soon as McFadden obtained these new wheats and before they were named, he generously supplied seed to all breeders interested. It was soon evident, as published by several workers at about the same time (Clark and Ausemus 1928, Goulden, Neatby, and Welsh 1928) that the type of resistance carried by Hope and H44 was simply inherited in crosses with susceptible varieties of vulgare. Resistance is dominant in F_1, and segregation in F_2 and later generations has been found to be dependent upon one or two pairs of factors. From crosses studied by Pan (1940) it seems probable that resistant lines obtained from crosses with H44 carry the same factors for resistance as Hope. In studies of F_3 lines from Hope and H44 crosses with stem-rust-susceptible varieties of vulgare, however, numerous workers have found a rather wide variation in types of segregation including ratios of resistant to susceptible of 9:7, 3:1, 15:1, 1:3, and 1:15 (Ausemus 1934, Churchward 1931, 1932). The important fact for the breeder is that resistant lines continue to breed true for resistance in later generations.

Bunt Resistance.—Farrar, in Australia, as early as 1901, reported studies in the breeding of wheat varieties resistant to bunt (*Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kuhn). Gaines, in Washington, has made extensive studies of inheritance of bunt reaction. He classified his material as immune, resistant, intermediate, and susceptible. In crosses of resistant and susceptible varieties, susceptibility was dominant, but when immune varieties were used as one parent, there was a dominance of immunity in F_1. Although Gaines was unable to place his results on a simple factorial basis, he found it possible to select homozygous bunt-immune and bunt-resistant lines.

Briggs, working with nearly immune types of bunt resistance, determined the genetic constitution of 10 bunt-resistant varieties.

The Martin factor is completely dominant, whereas the Turkey and Hussar factors, when heterozygous, give an intermediate reaction (Briggs 1933). There is some evidence of modifying factors and Churchward (1931, 1932) has reported that the bunt resistance of Florence is due to a single recessive factor. Recently Briggs (1940) has concluded that there is a linkage between the Martin and Turkey factors with a recombination value of 34.22 per cent.

TABLE 11.—THE GENETIC CONSTITUTION OF 10 BUNT-RESISTANT VARIETIES OF WHEAT (AFTER BRIGGS 1934)

Variety	Bunt-resistant Factors
Martin	*MM hh tt*
White Odessa	*MM hh tt*
Banner Berkeley	*MM hh tt*
Odessa	*MM hh tt*
Sherman	*MM hh tt*
Hussar	*MM HH tt*
Selections 1418 and 1403	*mm HH tt*
Turkey 1558	*mm hh TT*
Turkey 3055	*mm hh TT*
Oro	*mm hh TT*

Other Problems of Disease-resistance.—Considerable information is available regarding inheritance of resistance to other diseases and insect pests, including reaction to scab, *Helminthosporium* sp., black chaff, mildew, leaf rust, stripe rust, and Hessian fly. Varieties differ widely in their mode of reaction, and for most of these pests it seems feasible to breed resistant varieties. In many cases, however, sufficient information is not available to place results on a genetic-factor basis.

Quantitative Characters.—It seems reasonable to conclude that all characters of crop plants are conditioned by genetic factors. Yield of grain is a complex character that results from the inheritance of genetic factors and their interaction under particular conditions of environment. What is inherited is manner of reaction under particular conditions and not the character itself. Yield of grain is the end result of vigor of plant, as expressed in number of heads, number of kernels per spike and spikelet, and size of individual kernel. Anything that interferes with the normal development of the plant, including injury from diseases and unfavorable environmental conditions, affects yield. The usual method adopted by the breeder, when quantitative characters are concerned, is to select parents of good yielding ability with desirable characters, including those particular qualities for which the crop is used, select during the segregating generations for the characters desired, and test hybrids for yielding ability and other characters before deciding which is the more desirable.

CHAPTER X

INHERITANCE IN OATS

Cytological studies made by Kihara, Nishiyama, and others place *Avena* species in three groups, based on differences in chromosome numbers. These have been summarized by Stanton (1936):

Group 1. $n = 7$ chromosomes. *Avena brevis* Roth (short oat); *A. wiestii* Steudel (desert oat); *A. strigosa* Schreb. (sand oat) and *A. nudibrevis* Vav. (small seeded naked oat).

Group 2. $n = 14$ chromosomes. *Avena barbata* Pott (slender oat) and *A. abyssinica* Hochst. (Abyssinian oat).

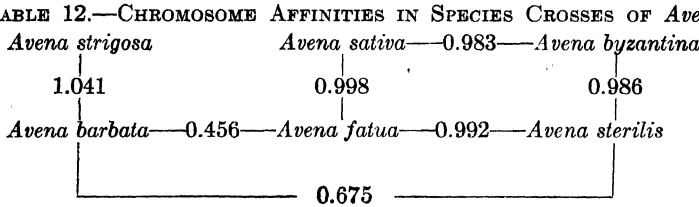
Group 3. $n = 21$ chromosomes. *Avena fatua* L. (common wild oat); *A. sativa* L., including *A. orientalis* Schreb. (common white or northern oat); *A. nuda* L. (hull-less oat); *A. sterilis* L. including *A. ludoviciana* Dur. (wild red or animated oat); and *A. byzantina* C. Koch, including *A. sterilis algeriensis* Trabut (cultivated red oat).

Kihara and Nishiyama (1932) have reported extensive studies of species crosses. Interspecific crosses between species belonging to the same chromosome group can be made easily. Crosses between the 14 × 28 chromosome species are relatively easy but produced fertile seeds only when the 28-chromosome species was the female. Crosses between the 14- and 42-chromosome species are difficult, and only a few successful crosses have been obtained. Viable seeds were produced only when the $n = 21$ species was the female. Reciprocal crosses between the 28- and 42-chromosome species gave well-developed kernels that germinated well.

Chromosome affinities in species crosses have not been completely worked out. A table by Nishiyama (1929) summarized some relationships by listing the number of bivalent associations inclusive of trivalents in hybrids between different species. He says, "If two parents differ in chromosome numbers, as many bivalents as the lower chromosome number of one parent may be expected. A full affinity between two species is, therefore, represented as 1.000 and no affinity as 0.000."

These results indicate that *A. barbata* is not closely related to *A. fatua*. *A. strigosa* ($n = 7$) when crossed with *A. barbata* ($n = 14$) showed more than seven bivalents, due possibly to autosyndesis of barbata chromosomes.

TABLE 12.—CHROMOSOME AFFINITIES IN SPECIES CROSSES OF *Avena*



As would be expected, crosses between species with different chromosome numbers are partly or nearly completely sterile because of irregularities of chromosome behavior during meiosis in the F_1 hybrid.

In the first-division metaphase of crosses between species that differ in chromosome numbers, bivalents and trivalents form a normal equatorial plate with the univalents scattered throughout the cell. Univalents divide equationally and pass to the poles, being included in the daughter nuclei, except for a few lagging chromosomes. The second division is irregular, since the univalents that have already divided equationally pass to the poles at random. There are many lagging chromosomes. In a cross between *A. barbata* with *A. strigosa*, 7 bivalents inclusive of trivalents are found commonly, and in some cases 8 or 9 bivalents. Trivalents are frequent. In crosses between *A. barbata* with *A. fatua*, the number of bivalents varied from two to eleven, with 1 to 4 trivalents; in the F_1 crosses of *A. barbata* × *A. sterilis*, 7 to 13 bivalents were found, inclusive of 0 to 4 trivalents. These results are not widely different from those in species crosses in wheat and give some reason for the belief that desirable characters from species with lower chromosome numbers can be transferred to the cultivated species with 42 chromosomes.

Cultivated varieties of oats belong chiefly to the species *A. sativa*, including the side-oat group *A. sativa orientalis*, commonly believed to have been derived from the wild oat (*A. fatua* L.) and the red oat varieties of *A. byzantina*, derived from the wild red oat, *A. sterilis*. Crosses between different species belonging to the 42-chromosome group are highly fertile, although there is

some evidence of abnormal chromosome behavior possible because of structural changes in one or more chromosomes within the various sets. This may cause trivalent, quadrivalent, or lagging chromosomes at meiosis. Nishiyama (1929) concludes, "All hexaploid hybrids have normal bivalents in the majority of P.M.C. at the metaphase of the first division. Sometimes 1–4 univalents and certain chromosome complexes are found together with normal bivalents. These irregularities are probably caused by mating between semihomologous chromosomes, not being normal partners."

Inheritance of Characters in Crosses between 42-chromosome Species.—Surface (1916), Philp (1933), and others have studied the inheritance of characters in crosses between *Avena fatua* and *A. sativa.* Characters associated with the fatua base on the grain of the lower floret that are completely correlated in inheritance include (1) heavy awn on the lower grain, (2) awn on the upper grain, (3) fatua base on the upper grain, (4) pubescence on the rachilla of the lower and upper grain, (5) pubescence on all sides of the lower grain and on the base of the upper grain. Philp explained these results by a factor *C* carried by the *fatua* parent that was partially dominant to *c* carried by *A. sativa.* It was suggested by Philp that the chromosomes carrying *C* in *A. fatua* and *c* in *A. sativa* were not entirely homologous and that the factor pair *Cc* responsible for a group of linked characters was inherited as a group complex. A partial lack of chromosome homologies was given as the probable reason for the complete linkage of the several character pairs.

The upper grains of the floret are persistent to their rachillas in *A. sterilis* and *A. byzantina,* which differentiates them from *A. fatua* and *A. sativa,* whereas cultivated varieties of *A. byzantina* differ from varieties of *A. sativa* in that there is a well-defined deep, oval cavity or "sucker mouth" on the base of the lemma of *A. byzantina.* These differences were illustrated in Chap. II.

Different investigators, including Fraser (1919), Hayes, Moore, and Stakman (1939), and Torrie (1939), have studied linkage relations of differential characters in crosses between varieties of *A. sativa* with *A. byzantina.* Coffman, Parker, and Quisenberry (1925) studied variability in Burt oats, belonging to *A. byzantina,* with particular reference to the following characters, using three characters in their classification:

Spikelet disarticulation, or the separation of the lower floret of the oat spikelet from the axis of the spikelet, was divided into three groups: (1) abscission, leaving a well-defined cavity in the face of the callus on the base of the lemma of the lower grain, (2) disarticulation by fracture, resulting in a rough fractured surface with little or no cavity in the base of the lemma, characteristic of *A. sativa*, (3) disarticulation by semiabscission, more or less intermediate between 1 and 2. Groups 1 and 3 characterize varieties of *A. byzantina* and homozygous segregates of the byzantina type from *A. sativa* × *A. byzantina*.

Floret disjunction, or the separation of the second or upper floret from the lower, was also classified in three groups: (1) disjunction by basifracture, the rachilla segment breaking near its base and remaining firmly attached to the upper floret, (2) disjunction by disarticulation at the apex of the rachilla segment, the rachilla segment remaining attached to the lower floret, the normal method in *A. sativa*, and (3) disjunction by heterofracture, the break occurring more or less intermediate between (1) and (2).

Basal hairs refer to conspicuous bristles on the base of the lower floret. Three classifications are given: (1) abundant long, (2) abundant mid-length, and (3) few.

In a series of crosses between Bond, *A. byzantina*, and cultivated varieties of *A. sativa*, several workers have found linkages for various character pairs. The linkages for the following characters were given by Hayes, Moore, and Stakman (1939).

1. Spikelet disarticulation and basal hair development, recombination value 2.7 per cent.

2. Floret disjunction (one of two genes involved) and basal hair development, recombination value 24.0 per cent.

3. Spikelet disarticulation and floret disjunction, recombination value 25.7 per cent.

Torrie (1939) observed linkage relations, in crosses between *A. sativa*, Iowa 444, and *A. byzantina*, Bond, for character differences including spikelet disarticulation, floret disjunction, rachilla attachment, basal hair length, awning, and red lemma color. The exact linear order of the genes was not accurately determined. The results indicated, however, that it was possible to obtain new combinations of these characters if desired.

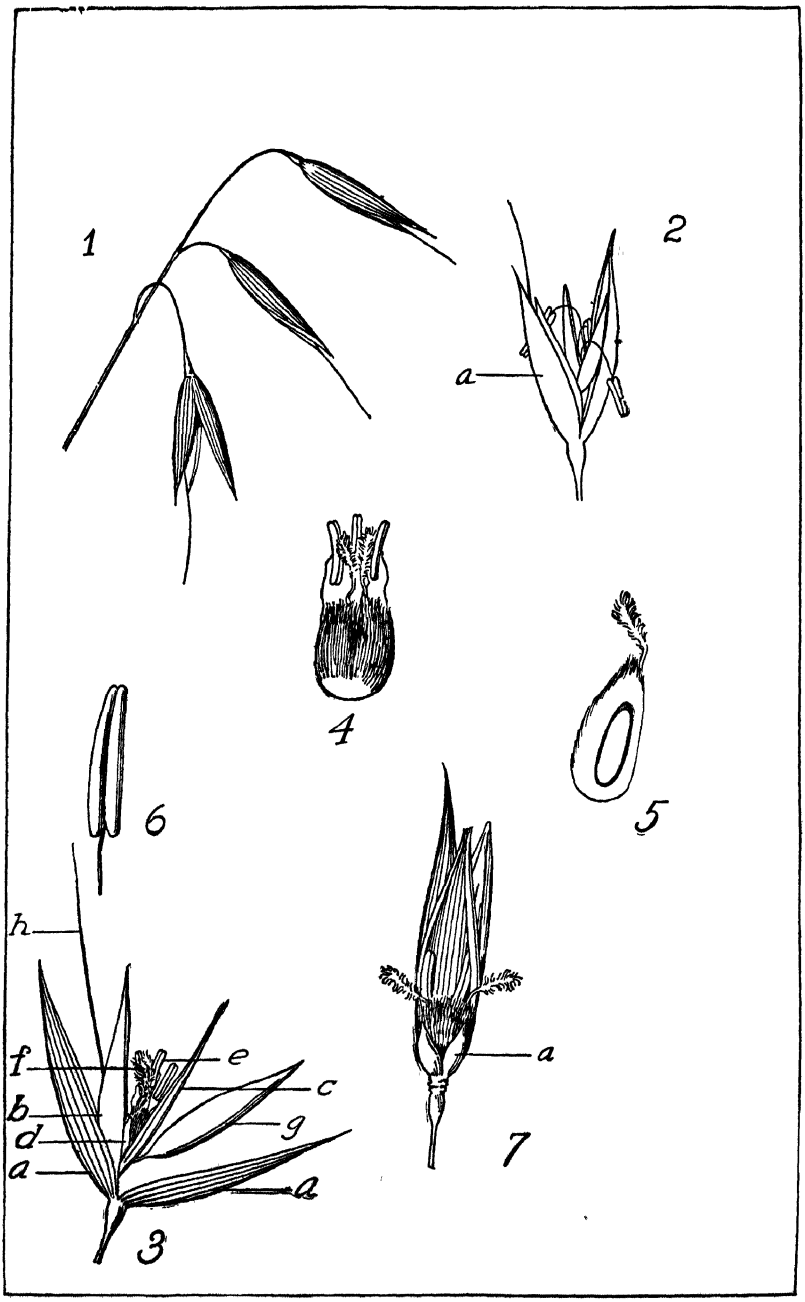
Differences in Awn Development.—Varieties of oats differ widely in awn development, both in the number of awns on the

upper and lower florets and in the degree of development of awns. The extent of awn development in a pure line varies rather widely from plant to plant and from one panicle to another on the same plant because of environmental conditions. Under uniform conditions, pure lines may be selected that show a range in awn development from nearly awnless to lines that have strongly developed awns on both the upper and lower florets. In some crosses, 3:1 or 1:2:1 ratios have been obtained when the separation is made into the larger groups, although, as a rule, minor modifying factors that modify the degree of development of the main factor pair are also involved.

Nilsson Ehle (1911*b*) and Love and Craig (1918*b*) found evidence that the gene for yellow lemma color inhibited the development of awns. Fraser (1919) studied a cross between Sixty-day, with yellow grains and no awns, with Burt, *A. byzantina*, with weak awns on the lower floret and frequently on the upper. In F_2, there was a ratio of fully awned (like Burt) to awnless of 1:3. The fully awned plants bred true in F_3. The degree of development of the awns ranged from weak awns like Burt to strong awns that were stiff and long, the strong awn being sharply twisted at the base, with a sharp bend about three-eighths of the way from the base to the tip. In crosses between Iogold, with weak to intermediate development of awns and with less than 50 per cent of the lower florets bearing an awn, and Bond, with 100 per cent weak awns in the lower florets, a range in F_2 from strong to weak awns was obtained and also a range from 25 per cent awned to fully awned (Hayes *et al.* 1939).

Color of Grain.—The color of the lemma has been classified as black, brownish red, gray, yellow, and white. Intensity of color is influenced by environmental conditions, and it is sometimes difficult to differentiate yellow and white. With bright sunshine during the later stages of development, the intensity of color is deeper than when wet, cloudy weather conditions prevail.

Black is epistatic to gray and yellow (Nilsson-Ehle 1909, Surface 1916), (Love and Craig 1918*b*), and gray is epistatic to yellow. Black vs. colorless, gray vs. colorless, and yellow vs. colorless segregated on a single factor basis in some crosses. In other crosses, there may be duplicate factors for black and for yellow. In a cross of Sixty-day, which produces yellow grain, with Burt, which produces brownish yellow, Fraser (1919)



For descriptive legend see opposite page.

obtained a ratio of 48 red:15 yellow:1 white in F_2. Apparently Burt carries a factor for red, *R*, and for yellow, *Y*, red being epistatic to yellow. The factor for yellow in Sixty-day is independent in inheritance of the factor for yellow carried by Burt. In crosses of Bond, reddish yellow × Iowa 444, colorless, Torrie (1939) concluded that the Bond parent carried two dominant factors, one for reddish color and one for yellow, that were independently inherited. Philp (1933) concluded that black and gray were independently inherited.

Hulled vs. Hull-less.—The *Avena nuda* species has been differentiated on the basis of its hull-less condition. Love and McRostie (1919), in crosses of hulled × hull-less, obtained an intermediate condition in F_1 and a ratio in F_2 of 1:2:1. Some evidence was given of a factor that modified the percentage of hulled grains on heterozygous plants. Philp (1933) obtained some hull-less F_2 plants in crosses of *A. sativa* × *A. fatua*, although they were not completely hull-less. He reports *A. nuda* plants from crosses of the *A. sativa* varieties made by W. Robb. The results can be explained by supposing that *A. sativa* carries two types of chromosome complexes, called *Z* and *z*, with *Z* epistatic to *z*. The *Z* complex carries a factor for hulled while *z* carries a factor for naked. A change of pairing whereby *Z* occasionally pairs with *z* will lead to the production of hull-less plants.

Spreading vs. Side Panicle.—Nilsson-Ehle explained a cross between spreading vs. side-panicle varieties on the basis of duplicate factors, either factor in the dominant condition producing an open panicle. Gaines (1917) and Garber (1922) found it difficult to separate spreading and side-panicle forms in segregating generations. Either 3:1 or 15:1 ratios would be expected in later generations if duplicate factors were involved from crosses of spreading vs. side-panicled varieties. There

FIG. 18.—Panicle and floral structure of oats.

1. Branch of oat panicle.
2. Spikelet, showing tertiary floret just after blooming: (*a*) primary floret.
3. Spikelet, showing floral parts: (*a*) outer glume; (*b*) flowering glume; (*c*) palea; (*d*) lodicules; (*e*) anther; (*f*) stigma; (*g*) secondary floret; (*h*) awn.
4. Outer parts removed, showing sexual organs.
5. Longitudinal section of ovary.
6. Anther.
7. Showing outer and flowering glume of lower spikelet removed: (*a*) lodicules, and sexual organs.

Size: 1, 2, about ×; 3, about 2×; 4, 5, 6 greatly enlarged; 7, about 2×.

probably are modifying factors that make the separation between open and side panicle difficult in some crosses.

Pubescence.—Cultivated varieties of sativa oats differ in the amount and in the presence of basal hairs on each side of the callus of the lower floret. One or two pairs of factors are involved in various crosses. Transgressive segregation, therefore, occurs in some crosses, forms being obtained in F_2 that are more pubescent than either parent or that lack pubescence. Pubescence on the back of the lower grain, the wild type of *A. fatua*, is dominant to the glabrous condition and may be controlled by one or two duplicate factors. One of these is closely linked and in some crosses completely linked with a factor for black grain color (Nilsson-Ehle 1909, Surface 1916, Love and Craig 1918*b*, and Philp 1933).

DISEASE REACTIONS

Three important diseases of oats are stem rust, *Puccinia graminis avenae* Eriks. & Henn., crown rust, *P. coronata* Corda, and the smuts, *Ustilago avenae* (Pers.) Jens. and *U. levis* (K. & S.) Magn.

Physiological specialization occurs for all three diseases. Dickson (1939) listed 9 physiologic races of stem rust and 44 of crown rust; Reed (1940) listed 29 of *U. avenae* and 14 of *U. levis*. In a breeding program, it is essential to use physiologic races prevalent in the locality to produce the artificial disease epidemic.

Stem Rust.—Varieties of oats are available that are resistant to several of the races common in the sections where stem rust frequently causes severe injury to susceptible varieties. Iogold and Rainbow are resistant to races 1, 2, 3, 5, and 7, and White Russian and derivatives are resistant to races 1, 2, 5, 8, and 9. Resistance to the five races 1, 2, 3, 5, and 7, to the three races 1, 2, and 5 and probably 8 and 9, and susceptibility to all five races form an allelic series (Smith 1934), and in any one cross the only homozygous types that can be obtained are the parental types. The resistance of Iogold and Rainbow under both field and greenhouse conditions to the races to which Iogold, Rainbow, and White Russian are resistant is of somewhat higher type than that of White Russian.

Welsh (1931) pointed out that resistance of Hajira to races 1, 2, 3, 5, and 7 was governed by the same factor pair. Joanette

is resistant to race 4, and in crosses with Hajira, segregation for rust reaction to race 4 was on the basis of 9 resistant: 7 susceptible. From a test of 21 lines breeding true for resistance to race 4, about half of these were resistant also to races 1, 2, 3, 5, and 7. Welsh (1937) has reported obtaining strains resistant to race 6 from crosses of Hajira with Joanette and explains the results on the basis of transgressive segregation. It is of some interest

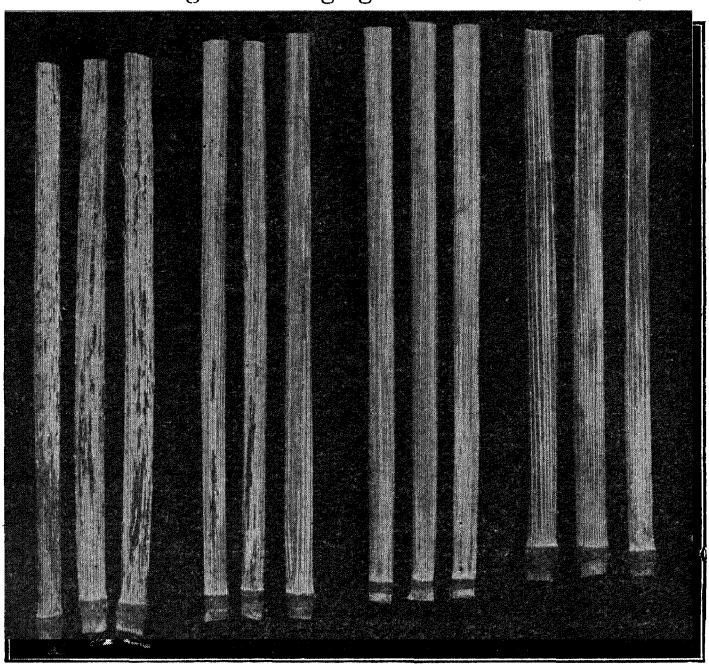


FIG. 19.—Culms of resistant and susceptible varieties of oats. From left to right: Victory, susceptible to stem rust; a susceptible F_2 plant of Victory × White Russian; a resistant F_2 plant of Victory × White Russian; resistant White Russian.

that White Russian was semiresistant to race 6 under field conditions in trials made by Welsh.

Although resistance to all races is to be desired, resistance of Rusota and Rainbow to races 1, 2, 3, 5, and 7 and of White Russian, Anthony, and Minrus to races 1, 2, 5, and perhaps 8 and 9 has protected these varieties from serious injury from stem rust under Minnesota conditions for many years when susceptible varieties such as Victory are often severely injured.

Crown Rust.—Although some varieties have been available that in some seasons show resistance to crown rust, the intro-

duction of Victoria from South America and Bond from Australia (Stanton & Murphy 1933) has furnished a basis for the breeding of resistant varieties, since both Bond and Victoria are resistant to many races of crown rust.

Using Victoria as one parent in crosses with susceptible varieties, Smith (1934) concluded that resistance was a partial dominant in F_1. Variable infection made it impossible to decide the number of factors involved. Stanton (1936) indicates a single factor pair with resistance dominant.

In crosses of Bond with susceptible varieties, Hayes *et al.* (1939) concluded that two pairs of factors were involved and placed segregation on a 9:7 basis, whereas Torrie (1939) explained crown-rust inheritance in crosses of Bond with Iowa 444 on the basis of two pairs of factors, *Ss* for resistance vs. susceptibility and *Ii*, a factor pair that in the dominant condition partly inhibited the effect of *S*.

Correlated Inheritance of Reaction to Three Diseases.—Stanton *et al.* (1934) obtained selections from crosses of Victoria × Richland that were resistant to the three diseases, and Murphy, Stanton, and Coffman (1936) reported selections from crosses where Bond was used as one parent that were resistant also to the three diseases. Hayes *et al.* (1939) and Torrie (1939) in crosses of Bond with varieties of *Avena sativa* found that reaction to all three diseases was inherited independently and found no evidence of association of reaction to the three diseases and other characters differentiating *A. byzantina* and *A. sativa*.

Smuts.—Reed (1940) summarized the reaction of a considerable group of varieties and species of oats to all physiologic races of both species of smuts. Markton, a well-known variety, Navarro (Stanton 1933), and Victoria are resistant to all races of both smuts. Black Mesdag has been used extensively in crosses and is resistant to all races of *Ustilago avenae* and resistant to 10 of the 14 races of *U. levis*. So far as tested, *A. barbata* is susceptible to all races of both smuts. A few varieties of oats are susceptible to most races. Canadian, for example, is susceptible to 28 races of loose smut and 13 of covered, being resistant to one race of each.

In crosses of Monarch selection × Black Mesdag (Stanton, Reed, & Coffman 1934), inoculated with races of *U. avenae* from Missouri, resistance was a dominant, and segregation on a 3:1

basis occurred. Crosses of Markton × Black Mesdag inoculated with *U. avenae*, where both parents were resistant, gave some susceptible F_3 progenies.

Hayes *et al.* (1928) studied crosses of Black Mesdag with other *A. sativa* selections, using a mixture of smuts for inoculation, and obtained results indicating that the Black Mesdag resistance to both smuts was due to the action of two pairs of factors, *R* for high resistance and *I* for immunity carried by Black Mesdag. A selection from this cross that has proved highly resistant to a mixture of races of smuts at University Farm, St. Paul, Minnesota, was crossed with Bond, resistant also to the races used. Some susceptible plants and lines occurred in F_2 and F_3, respectively. In crosses of Bond with susceptible varieties, resistance was dominant and the segregation was on a 3:1 basis. Results of this nature are common in polyploids of an amphidiploid nature where cases of duplicate or triplicate factors that condition the development of a character are of relatively frequent occurrence.

QUANTITATIVE CHARACTERS

Many characters of oats of interest to the breeder are undoubtedly due to the interaction of multiple factors. These include such characters as data of maturity, height of plant, resistance to lodging, number of culms, winter hardiness, drought resistance, percentage of hull, weight per bushel, and yielding ability.

It is important for the breeder to analyze the varieties that are used as parents for all characters of importance and select during the segregating generations, under controlled conditions when possible, for the characters desired.

CHAPTER XI

INHERITANCE IN BARLEY

CLASSIFICATION AND GENETICS OF BARLEY SPECIES

Harlan (1918) classified barley into four species, essentially on the basis of fertility of the lateral spikelets. The following key is taken from Harlan's paper:

All spikelets fertile (6-rowed barley):
  Lemmas of all flowers awned or hooded..... ..*Hordeum vulgare* L.
  Lemmas of lateral flowers bearing neither awns nor hoods . ...
  .....*H. intermedium* Kcke.
Only the central spikelets fertile (2-rowed barley):
  Lateral spikelets consisting of outer glumes, lemma, palea, rachilla, and usually rudiments of the sexual organs.....*Hordeum distichon* L.
  Lateral spikelets reduced, usually to only the outer glumes and rachilla, rarely more than one flowering glume present, and never rudiments of sexual organs.....*Hordeum deficiens* Steud.

The *H. intermedium* group would be classified more accurately by the statement: Central spikelets fertile, lateral spikelets partially fertile.

A single-factor difference for type of head is found in crosses of some varieties of *H. vulgare* × *H. deficiens; H. vulgare* × *H. distichon;* and *H. distichon* × *H. deficiens.* Engledow (1924) and Hor (1924) concluded that an allelic series of factors differentiated the type of lateral florets found in these three species.

In some crosses of varieties of *H. vulgare* with *H. distichon,* as has been already mentioned, a segregation of two-rowed (*VV*):intermediate (*Vv*):six-rowed (*vv*) of 1:2:1 is obtained. In cases of monohybrid segregation, the lateral florets are usually infertile but will always be awn-pointed. In other crosses of *H. vulgare* with *H. distichon,* seven classes may be differentiated by the breeding behavior in F_3. Harlan and Hayes (1920) gave the first complete genetic analysis of the results from such crosses, explaining the results on the basis of two factor pairs. Robertson (1933) obtained similar results. Both obtained true-

breeding intermedium types in F_3. The lateral spikelets in the intermedium obtained by Harlan and Hayes (1920) were partially fertile, varying from 18 to 55 per cent in different F_3 lines. In the intermedium obtained by Robertson (1933), the lateral spikelets were infertile, *i.e.*, less than 2 per cent fertile. Leonard



FIG. 20.—Heads of the cultivated species of barley. From left to right, *Hordeum vulgare*, *H. intermedium* (fertile), *H. intermedium* (infertile), *H. distichon*, *H. deficiens*.

(1940) found that the fertile, infertile, and nonintermedium types were differentiated by genes belonging to a multiple-allelic series, designated as I^hI^h, *II*, and *ii*, respectively.

Intermedium barley can be classified on the basis of the rounded lemmas of the lateral florets, which are never awn-pointed. This condition is expressed only in the presence of *VV*. In the presence of *Vv*, the lateral florets are always awn-pointed.

These are designated intermediates. Varieties that are genotypically *vv* are six-rowed, with complete fertility of the lateral florets. These may be *vv* I^hI^h, *vv II*, or *vv ii*.

The genotype of an unknown six-rowed variety for the intermedium series may be determined by crossing it with a tester strain of known genotype, such as Nigrinudum, which is known to be *VV II*. The term infertile intermedium may be used when less than 2 per cent of the lateral spikelets are fertile and the term fertile intermedium used to designate those types with more than 2 per cent (usually 10 to 60 per cent) of fertile lateral florets. The following scheme will illustrate how the genotypic constitution of the six-rowed variety may be determined when crossed with a variety that has the genotype *VV II*. The phenotypes of the F_1 and F_2 generations are given for crosses of *VV II* with three different homozygous six-rowed genotypes.

Phenotype of:		Genotype of six-rowed variety
F_1	F_2	
Fertile intermediate.....	Infertile intermedium, fertile intermediate, and 6-rowed in ratio of 1:2:1	*vv II*
Fertile intermediate.....	Infertile intermedium, fertile intermedium, fertile intermediate, and 6-rowed in ratio of 3:1:8:4. The distinguishing feature is the presence of fertile intermediums.	*vv* I^hI^h
Infertile intermediate.....	2-rowed, infertile intermedium, infertile intermediate, fertile intermediate, and 6-rowed in a ratio of 3:1:6:2:4. The distinguishing feature is the production of 2-rowed segregates but no fertile intermedium	*vv ii*

It is sometimes difficult to determine morphologically whether barley varieties are genetically of the distichon type (*VV ii*) or are infertile intermediums (*VV II*). To discriminate between them, they may be crossed to tester strains of the genotype *vv II*. If segregation is on a monohybrid basis, the genotype of the two-rowed parent is *VV II*, and the variety is an infertile inter-

medium. If a dihybrid segregation occurs, the two-rowed parent is a true two-rowed barley with the genotype *VV ii*.

The four species of barley described by Harlan (1918) are differentiated genetically by only two factor pairs and their alleles.

CHROMOSOME NUMBER IN GENUS *HORDEUM*

In the genus *Hordeum*, as in *Triticum* and *Avena*, the basic chromosome number is seven pairs. Multiples of this basic number are obtained also. Numerous investigators have reported the chromosome number of different species, and some of these are listed below:

7 pairs of chromosomes:
Hordeum bulbosum, H. deficiens, H. distichon, H. gussoneanum, H. hexastichum, H. intermedium, H. jubatum, H. murinum, H. nodosum, H. pusillum, H. spontaneum, H. vulgare
14 pairs of chromosomes:
H. bulbosum, H. jubatum, H. murinum, H. secalinum
21 pairs of chromosomes:
H. nodosum

The species *bulbosum*, *jubatum*, and *murinum* have been reported by different investigators as having either 7 or 14 pairs of chromosomes. *H. nodosum* has been reported as having 7 or 21 pairs. The economic species all have 7 pairs of chromosomes.

LINKAGE GROUPS

There are numerous characters in barley that are easily differentiated. Since the number of chromosome pairs is seven for each of the four cultivated species, barley has been used extensively in studies of linkage relations. More than one hundred different characters have been investigated. Robertson, Wiebe, and Immer (1941) summarized the known linkage information and suggested symbols to be used in designating the various characters. In Table 13 are given some of the characters that are known to be simply inherited and that have been placed in one of the linkage groups.

It is of some interest to note that to date the only four factor pairs known for group 6 involve lethal seedlings. In all other chromosomes, easily differentiated, completely viable characters are available.

Internode Length in the Rachis of the Spike.—Varieties of barley vary greatly in density of the head, as measured by length

TABLE 13.—SIMPLY INHERITED CHARACTERS IN DIFFERENT LINKAGE GROUPS

Character Differences	Symbol
Group 1:	
Non-6-rowed vs. 6-rowed	*Vv*
Red vs. white pericarp	Re_1re_1
Purple vs. white lemma	*Pp*
Purple vs. white straw	*Prpr*
Toothed vs. untoothed lemma	*Gg*
Awnless vs. awned	*Lklk*
Normal vs. albino seedlings	*Aa*
Normal vs. albino seedlings	A_3a_3
Normal vs. albino seedlings	A_4a_4
Green vs. chlorina seedlings	*Ff*
Green vs. virescent seedlings	*Yy*
Green vs. orange seedlings	*Oror*
Group 2:	
Black vs. white lemma and pericarp	*Bb*
Normal vs. "third outer glume"	*Trd trd*
Normal vs. albino seedlings	$A_t a_t$
Group 3:	
Hulled vs. naked	*Nn*
Normal vs. albino seedlings	$A_{c2} a_{c2}$
Dense vs. lax head	*Ll*
Group 4:	
Hooded vs. awned	*Kk*
Blue vs. white aleurone	*Bl bl*
Fertile intermedium, infertile intermedium, and nonintermedium	I^h, *I*, *i*
Group 5:	
Rough vs. smooth-awned	*Rr*
Long vs. short-haired rachilla	*Ss*
White vs. orange lemma	*Oo*
Normal vs. albino seedlings	$A_b a_b$
Red vs. white pericarp	*Re re*
Group 6:	
Green vs. xantha seedlings	$X_c x_c$
Green vs. xantha seedlings	$X_s x_s$
Green vs. albino seedlings	$A_c a_c$
Green vs. albino seedlings	$A_n a_n$
Group 7:	
Normal vs. brachytic	*Br br*
Green vs. chlorina seedlings	$F_c f_c$
Green vs. virescent seedlings	$Y_c y_c$
Resistance vs. susceptibility to *Puccinia graminis*	*Tt*

of the internodes of the spike. The density varies comparatively little from year to year. Hayes and Harlan (1920) studied the mode of inheritance of internode length in five crosses. In two crosses, a single-factor-pair difference explained the results satisfactorily. Short internode length was dominant in one of these crosses, but the head density in the second cross was intermediate in F_1. In another cross, a broad difference of two factor pairs was indicated by the segregation in F_2 and F_3.

In the cross of Hanna × Zeocriton (see Table 14), lax and dense varieties, respectively, the F_2 ranged from above the modal

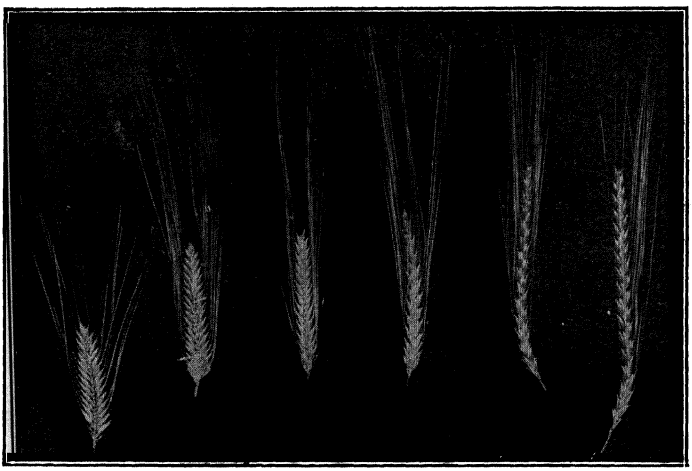


FIG. 21.—Average spikes of the Zeocriton (left), Hanna (right), and four homozygous lines. Mean densities are as follows: Zeocriton, 1.9 mm.; Hanna × Zeocriton, 448-1, 2.3 mm.; 448-5, 2.9 mm.; 448-11-3, 3.7 mm.; 448-16, 4.3 mm.; Hanna, 4.6 mm. (*After Hayes and Garber*, 1927.)

class of Hanna to the modal class of Zeocriton, even though only 141 individuals were studied. F_3 families were grown from F_2 plants representing different densities. Progenies from selected plants in certain F_3 lines were tested further in F_4. Some F_3 lines bred comparatively true, the range for density being no greater than for the parental varieties. Other F_3 lines were as variable as the F_2 generation and still others more variable than the parents but less variable than the F_2. Typical heads of the parents and segregates from homozygous lines are illustrated in Fig. 21.

Homozygous lines differing in density were obtained in F_3 and F_4. The homozygotes appeared to fall in groups. The general

TABLE 14.—HANNA (460) × ZEOCRITON (1039) = 448 (AFTER HAYES AND GARBER, 1927)

Variety	Generation	Year	Density of parent	Class centers for progeny density in mm.																			Total	Mean	C.V.
				1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6			
Hanna	P_1	1916	.....											6	13	30	10	12	9	2	1				83	4.1 ± 0.02	7.5 ± 0.4
	P_2	1917	3.6–4.9											1	9	39	61	36	20	1					167	4.4 ± 0.01	5.0 ± 0.2
	P_3	1918	4.1–4.7												1	3	26	63	60	36	15	4		1	209	4.6 ± 0.01	5.7 ± 0.2
Zeocriton	P_1	1917	.....		16	18																			34	1.9 ± 0.01	4.8 ± 0.5
	P_2	1918	.....	5	53	72	22	1																	153	2.0 ± 0.01	7.7 ± 0.3
Hybrid 448	F_2	1916	.....			4	8	9	18	18	17	18	12	9	5	13	7	2		1					141	3.0 ± 0.04	23.1 ± 1.0
448-1	F_3	1917	2.1		5	19	18	6																	48	2.1 ± 0.02	8.1 ± 0.6
448-1 (4 plants)	F_4	1918	1.9–2.4		1	21	85	79	26	1															213	2.3 ± 0.01	7.4 ± 0.2
448-2	F_3	1917	2.4	1	3	17	21	25	6	5	7	4	2												91	2 4 ± 0.03	15.9 ± 0.8
448-5	F_3	1917	2.7					4	16	10	5														35	2.7 ± 0.02	6.3 ± 0.5
448-5 (4 plants)	F_4	1918	2.7–3.0					3	52	111	104	38	1												309	2.9 ± 0.01	6.6 ± 0.2
448-7	F_3	1917	2.8			2	1		3	13	14	6													39	2.7 ± 0.03	10.2 ± 0.8
448-7-1	F_4	1918	2.0		3	22	56	20	6																107	2.2 ± 0.01	7.7 ± 0.4
448-7-3	F_4	1918	3.1						1	7	42	40	8	4											102	3.1 ± 0.01	5.8 ± 0.3
448-9	F_3	1917	3.0		3	1	4	7	6	7	8	4	1	2	2	1	3								49	2.9 ± 0.06	21.4 ± 1.5
448-9-4	F_4	1918	2.3		6	35	19	3																	63	2.1 ± 0.01	6.8 ± 0.4
448-9-16	F_4	1918	3.2			1	3	1	15	10	8	15	17	6	2	10	7	3	3	3					104	3.3 ± 0.04	19.5 ± 0.9
448-9-19	F_4	1918	3.2								1	5	5	3	1	1									16	3.4 ± 0.04	7.3 ± 0.9
448-9-30	F_4	1918	4.5											1	7	25	14	12							59	4.3 ± 0.02	4.7 ± 0.3
448-11	F_3	1917	3.4							1	6	15	23	8	5										58	3.4 ± 0.02	6.9 ± 0.4
448-11-2	F_4	1918	3.0							11	27	30	4	1											73	3.1 ± 0.01	5.5 ± 0.3
448-11-3	F_4	1918	3.7										5	19	16	5									45	3.7 ± 0.02	4.3 ± 0.3
448-13	F_3	1917	3.5							3	6	12	2	3	12	10	4	1							53	3.6 ± 0.04	12.0 ± 0.8
448-13-1	F_4	1918	3.1							1	9	18	4	6	19	31	24	7	6		1				126	3.8 ± 0.03	12.0 ± 0.5
448-13-2	F_4	1918	3.0							5	13	29	13	4											64	3.2 ± 0.02	6.3 ± 0.4
448-13-5	F_4	1918	4.2												3	21	23	8	1	1					57	4.2 ± 0.02	4.6 ± 0.3
448-16	F_3	1917	4.3											1	3	10	15	8	3						40	4.4 ± 0.02	5.0 ± 0.4
448-16 (4 plants)	F_4	1918	4.3–4.8										2	11	65	101	77	51	20	3		1			331	4.3 ± 0.01	6.3 ± 0.2
448-18	F_3	1917	4.0								1	3	3	1	2	11	7	5	4		1				38	4.0 ± 0.05	11.2 ± 0.9

nature of the results is illustrated by Fig. 22. The results could be explained on a genetic basis by the hypothesis that the parent varieties differed by three independently inherited factors. These factors were considered to have a cumulative effect. Other factors, having smaller effects, doubtless were present also and modified the expression of the main density factors.

Wexelsen (1934), from studies of six crosses involving five different varieties, found that internode length in different crosses

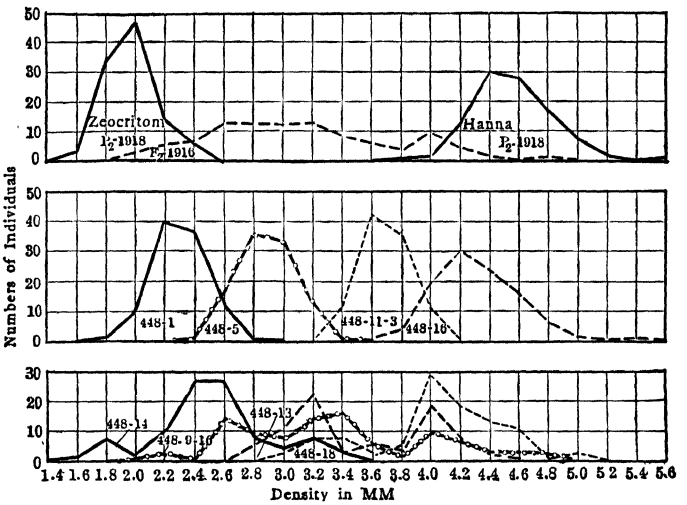


FIG. 22.—Diagrams showing the densities of parental forms and of the F_2 generation in a cross between the Zeocriton and Hanna barleys (upper), of four pure lines (middle), and of several heterozygous lines (lower). (*After Hayes and Harlan*, 1920.)

was differentiated by one to five factor pairs. A total of six factor pairs appeared to be involved in these crosses. These factors had different effects on internode length when heterozygous, some heterozygous types being intermediate, one being near the short, and another near the long internode parental type. One of the internode-length-factor pairs (L_2l_2) was found to be linked with rough vs. smooth awns (Rr) and long vs. short-haired rachilla (Ss), and another (L_4l_4) was linked with non-six rowed vs. six-rowed head type (Vv).

Reaction to *Helminthosporium sativum*.—The best proof that quantitative characters are inherited in the same manner as qualitative characters has been obtained from linkage studies. Quantitative characters may be correlated with qualitative

characters when the mode of inheritance and linkage relations of the qualitative characters are known. By means of such studies, it is possible frequently to determine the minimum number of genes controlling the quantitative character in crosses between known varieties. This mode of attack was used by Griffee (1925) in a rather extensive study of reaction to spot blotch *Helminthosporium sativum* P. K. & B.

The contrasted characters of the parent varieties were as follows:

Svanhals	Lion
White hull and pericarp	Black hull and pericarp
2-rowed (*distichon*)	6-rowed (*vulgare*)
Rough awn	Smooth awn
Resistant to spot blotch	Susceptible to spot blotch

Each of the character pairs, black vs. white, two-rowed vs. six-rowed, and rough vs. smooth awn, are known to be dependent upon single-factor differences and to be independently inherited. By considering each of these character pairs separately, a definite association was found in F_2 (each F_2 plant was tested by growing and examining its F_3 progeny), between each character pair and reaction to spot blotch. The nature of the results is illustrated in Fig. 23, in which the lower *Helminthosporium* figure indicates susceptibility and the higher figure, resistance to the disease. More resistant plants were found in the two-rowed group than in the six-rowed, in the white than in the black, and in the rough- than in the smooth-awned. It seemed fair to conclude that at least three factor pairs, or groups of factors, were involved in determining reaction to *H. sativum*, and these were located in the same chromosomes as the factors for color, row number, and smooth vs. rough awns. Resistance and susceptibility were not dependent upon the same factors that conditioned the other characters since it was possible to obtain a resistant, white-hulled, six-rowed, smooth-awned variety and also a resistant, black-hulled variety from the cross of Svanhals × Lion.

Reaction to Stem Rust.—Powers and Hines (1933) studied the reaction to stem rust *Puccinia graminis tritici* in crosses of Peatland × Glabron and Peatland × Minn. 462. Peatland was the resistant parent. Glabron and Minn. 462 are sister selections from a cross of Smooth Awn × Manchuria, and both are sus-

ceptible. Reaction to stem rust in the mature-plant stage was due to a single-factor pair with resistance dominant. Rust

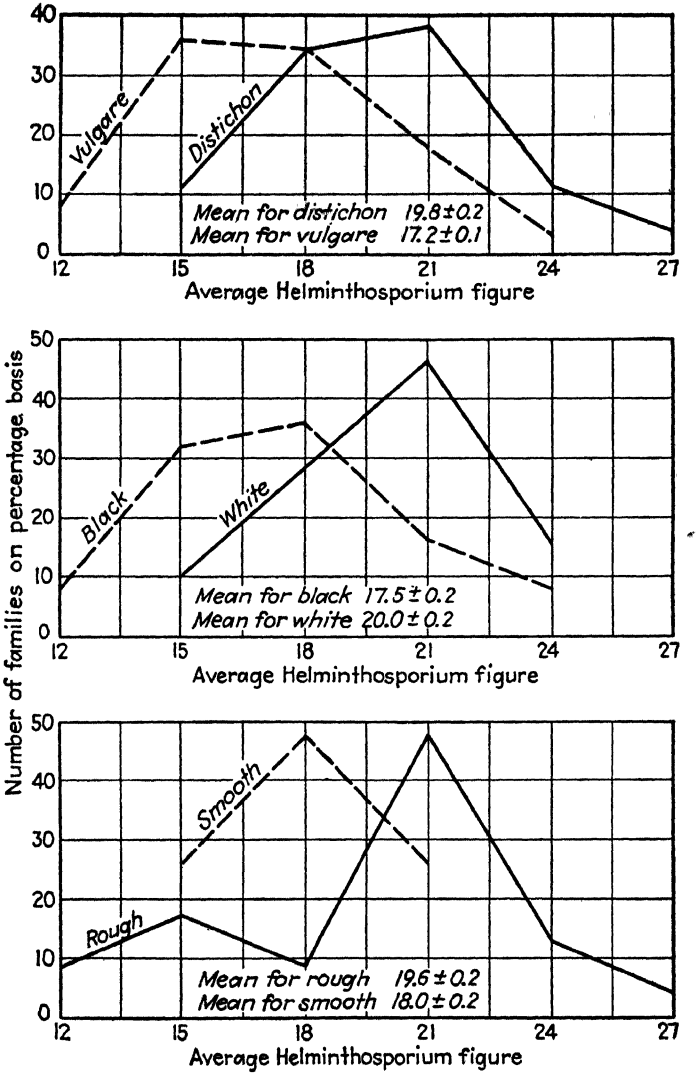


FIG. 23.—Distribution of Helminthosporium reaction of F_3 lines homozygous for other characters in cross of Svanhals × Lion. Lower figure for *Helminthosporium* reaction indicates susceptibility and higher figure resistance to the disease. (*From Griffee*, 1925.)

reaction was found to be independent of rough vs. smooth awns. Reid (1938) corroborated the foregoing conclusions regarding

reaction to a large collection of physiologic races of stem rust in the mature-plant stage in a cross of Barbless × Peatland.

Brookins (1940) found the factor pair for resistance vs. susceptibility to stem rust (*Tt*) to be located in the seventh linkage group, linked with normal vs. brachytic plant type (*Brbr*) and normal vs. chlorina seedlings (F_cf_c). The gene order was

$$\underset{t}{T} \quad 12.6 \quad \underset{br}{Br} \quad 9.8 \quad \underset{f_c}{F_c}$$
$$\underbrace{\qquad\qquad\qquad}_{16.7}$$

Brookins found also that reaction to physiologic races 19, 36, and 56 in the seedling stage, in crosses involving Peatland, was monohybrid, with resistance dominant. The same factor pair that differentiated seedling reaction to these three races also controlled reaction to a large collection of races in the mature-plant stage in the field. Apparently the type of rust resistance found in Peatland is physiological, since the same gene pair controlled the same reaction to rust throughout the life of the plants.

Resistance to Mildew.—Stanford and Briggs (1940) summarized the studies on inheritance of resistance to barley mildew (*Erysiphe graminis hordei*) carried out at the California Agricultural Experiment Station. From studies of the genetics of resistance to race 3 in 10 resistant varieties, crossed with one another and with susceptible Atlas, the factorial composition of the resistant varieties was as follows:

Variety	Factors for Resistance to Mildew
Hanna	$Ml_h\ Ml_h$
Goldfoil	$Ml_g\ Ml_g$
Arlington awnless	$Ml_p Ml_p\ Ml_y\ Ml_y$
Chinerme	$Ml_p Ml_p\ Ml_y\ Ml_y$
Nigrate	$Ml_p Ml_p\ Ml_y\ Ml_y$
Algerian	$Ml_a\ Ml_a$
S.P.I. 45492	$Ml_a\ Ml_a$
Kwan	$Ml_k\ Ml_k$
Psaknon	$Ml_p\ Ml_p$
Duplex	$Ml_h\ Ml_h\ Ml_p\ Ml_p\ ml_d\ ml_d$

Seven different factors for mildew resistance—six dominant and one recessive—were found. The number of resistant factors in a single variety varied from one to three.

The two different factor pairs differentiating Algerian and Kwan were found to be linked with 9.81 per cent recombination. The other five factors appeared to be independent of these two and independent of one another. Thus, seven different factors are involved in the control of a single physiologic race of a single disease. This appears to be the largest number yet located in any species of plants.

INTERACTION OF FACTORS AFFECTING QUANTITATIVE CHARACTERS

Quantitative characters are extremely important to the plant breeder. Studies of the genetics of these characters present serious difficulties, since the number of genes involved usually is large and the effect of single genes frequently is small. Information on the nature of interaction of factors affecting quantitative characters is very meager.

Powers (1936) studied the nature of the interaction of genes affecting the four quantitative characters, yield of seed per plant, number of spikes per plant, height of plant, and length of awn in a cross between varieties of *Hordeum deficiens* and *H. vulgare*. Single plants of the parents F_1 and F_2 were classified for black vs. white glumes (*Bb*), deficiens vs. vulgare type of spike (*Vv*), and normal vs. brachytic type of growth (*Brbr*). The yield of seed, number of spikes, plant height, and awn length were determined for individual plants. The genotype of the F_2 plants for the qualitative characters was determined from a progeny test in F_3.

Powers found that the homozygous black (*BB*) and homozygous white (*bb*) segregates did not differ significantly in the four quantitative characters measured. The heterozygotes (*Bb*) exceeded the two homozygotes in all four quantitative characters, although not significantly so in some comparisons. This increase in the *Bb* segregates in F_2 over the *BB* and *bb* may be explained as being due to favorable and at least partially dominant genes located in the chromosome pair carrying *Bb*.

Plants with the vulgare type of spike (*vv*) yielded more than those with the deficiens (*VV*) or the heterozygotes (*Vv*). The *Vv* segregates yielded more than the *VV* plants. Normal plants of the genotypes *BrBr* or *Brbr* were higher in yield than the brachytic plants (*brbr*)

In making comparisons of the differences in yield of seed between plants of the genotypes *vv Brbr* and *VV Brbr* with *vv brbr* and *VV brbr*, the cross difference (*vv Brbr* − *VV Brbr*) − (*vv brbr* − *VV brbr*) was positive and significant. It is apparent that the difference in yield between segregates of the vulgare type (*vv*) was greater in the presence of the nonallelic genes *Brbr* rather than in the presence of the less favorable *brbr* and of the deficiens type (*VV*). In general, it was found that genes favorable to high plant yield when transferred from a low to a high yielding geno-type of a nonallelic factor pair, in comparison with their alleles, were still more favorable to the development of grain yield than in the presence of the low yielding genotype.

The foregoing evidence is the reverse of that expected according to Rasmusson's (1935) interaction hypothesis, which assumes "that the effect of each factor on the genotype is dependent upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction." Rasmusson found support for his theory in a study of interaction of factors governing early and late maturity in *Pisum*. Powers (1934) in a study of factors governing habit of growth in *Triticum* obtained results that support this hypothesis also.

It is apparent that differences in interaction between genes controlling quantitative characters occur and that no general rule can be given at the present time that will describe all conditions. Powers concluded that at the present time any hypothesis regarding the nature of gene interactions is of doubtful value as a means of prediction.

CHAPTER XII

INHERITANCE IN FLAX

All cultivated varieties of flax belong to the species *Linum usitatissimum* L. The haploid chromosome number usually found is 15, although 16 in the haploid and 32 in the diploid have been reported by several investigators (Tammes 1928, Dillman 1936). Tammes gave the chromosome numbers of other Linum species as 8, 9, 10, 12, 14, 15, and 18 in the haploid condition. Extensive attempts to cross common varieties of flax with many of the wild-flax species have been made, but without success except in crosses between *L. usitatissimum* and *L. angustifolium*, which can be made without difficulty. The hybrids are completely fertile as a rule. Because *L. angustifolium* has the same number of chromosomes and crosses readily with the common species, it has been considered by Tammes as the probable ancestor of common flax. *L. angustifolium* differs from common cultivated varieties of flax in that the seeds and capsules are smaller, the edges of the partition walls of the capsule are hairy, and the capsules open or dehisce at maturity.

Hairy capsules were dominant to glabrous in F_1, and segregation in F_2 was on a 3:1 basis. Dehiscence of the capsule at maturity was imperfectly dominant over the closed type of capsule, and several factors were necessary to explain segregation in F_2. In a cross of cultivated varieties with a particular form of *L. angustifolium* having a strongly tillered and branched habit of growth of more delicate type of plant than cultivated varieties, Tammes found no plant among 300 grown in the F_2 generation that belonged strictly to the *L. usitatissimum* type. The length and width of petal and length of seed in *L. angustifolium* was less than that of the cultivated varieties. The inheritance of these character differences was dependent upon multiple (polymeric) factors.

There is a similar range of flower colors in *L. angustifolium* as in cultivated varieties, although of the factors involved in flower,

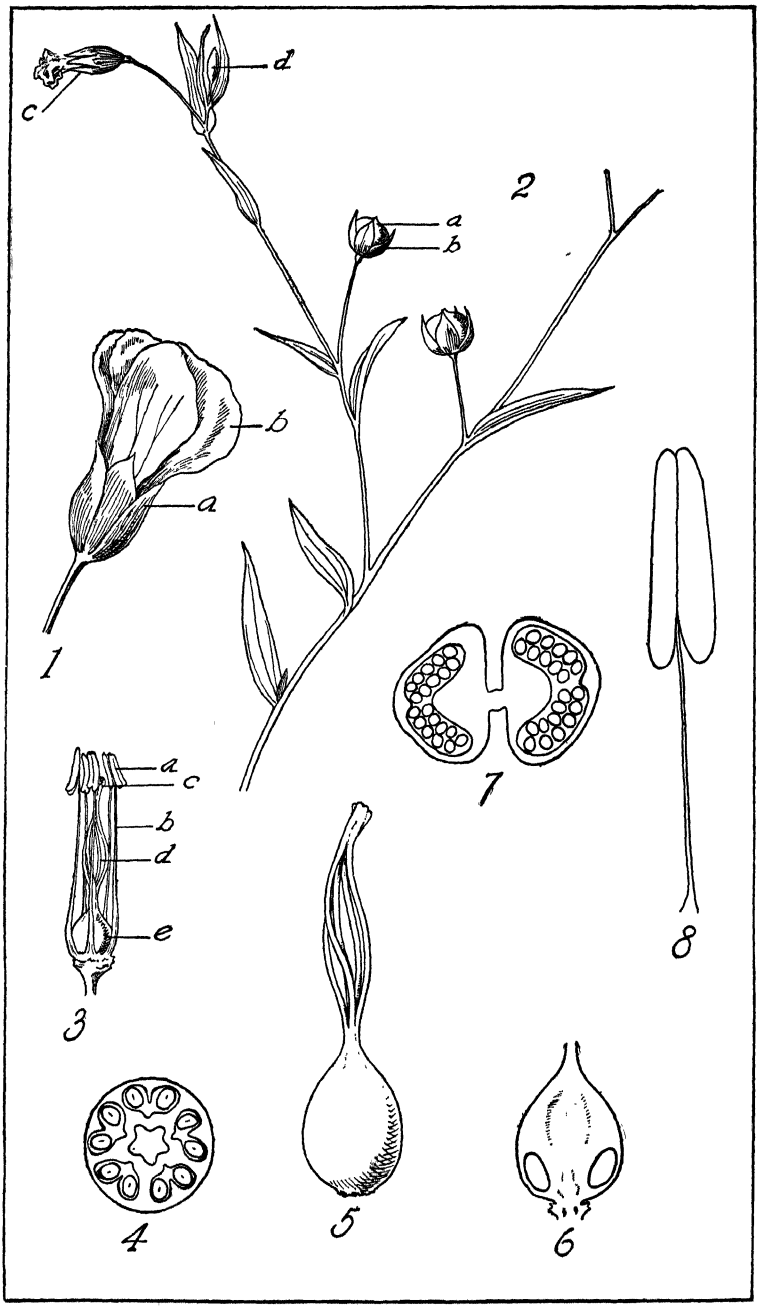


FIG. 24.—For descriptive legend see opposite page.

seed, and plant colors, only two were exactly the same in *L. angustifolium* as in *L. usitatissimum*.

Factors for Flower and Seed Color in Common Flax.—Dillman (1936) has summarized the major effects of the interaction of eight genes for seed and flower colors as determined by Tammes. The pure lines that he obtained from Tammes, their petal, anther, and seed colors and genetic composition are summarized in Table 15.

TABLE 15.—GENETIC COMPOSITION AND CHARACTERS OF PURE LINES OF FLAX (AFTER DILLMAN)

C. I. No.	Factor composition*								Description		
									Petals	Anthers	Seeds
765	*AA*	*B*$_1$*B*$_1$	*B*$_2$*B*$_2$	*C'C'*	*DD*	*EE*	*FF*	*HH*	Blue	Blue	Brown
766								*hh*	Blue	Yellow	Brown
768	*aa*								Light blue	Blue	Brown
769						*ee*		*hh*	Pale blue	Yellow	Brown
770							*ff*		Lilac	Blue	Brown
771	*aa*						*ff*		Light lilac	Blue	Brown
772					*dd*				Pink	Yellow	Brown
773					*dd*		*ff*		Deep pink	Yellow	Light brown
774				*c'c'*					White, flat	Blue	Brown
775		*b*$_1$*b*$_1$							White, crimped	Yellow	Greenish yellow
776			*b*$_2$*b*$_2$						White, crimped	Yellow	Brown
777		*b*$_1$*b*$_1$		*c'c'*					White, flat	Yellow	Greenish yellow
778				*c'c'*	*dd*				White, flat	Yellow	Grayish brown

* All dominant factors are present in common blue flax, C. I. 765, the recessive factors given being those actually determining the character differences from common blue.

These eight factors are believed by Tammes to be carried in different chromosomes. From the table it may be noted that B_1, B_2, and C' are basic color factors, all in the dominant condition being necessary for the production of color in the petals. The

FIG. 24.—Structure of the flowers of flax.

1. Single flower: (*a*) calyx; (*b*) corolla
2. Branch showing: (*a*) seed boll; (*b*) calyx; (*c*) flowers just after blooming; (*d*) bud.
3. Calyx and corolla removed to show sexual organs in position: (*a*) anther; (*b*) filament; (*c*) stigma; (*d*) one of 5 divisions of style; (*e*) ovary.
4, 6. Cross and longitudinal section of ovary.
5. Ovary, stigma, and 5-lobed style.
7. Cross section of anther.
8. Anther.

Size: 1, about 5 ×; 2, about × ; 3, nearly 4 ×; 4–8, greatly enlarged.

factors D and F determine the tint of the petals. When D is recessive, in the presence of the basic factors, the petal color is pink; F in a recessive condition causes lilac; and when both D and F are recessive, deep pink results. Factors A and E are intensifiers. When a or e are homozygous, recessive, the color is of a lighter shade.

B_1, B_2, C' and D influence the shape of the petals, all four factors being in a dominant condition in most common flax varieties with broad, flat petals. If either b_1 or b_2 are recessive, in the presence of both C' and D, the petals are narrow and "crimped," *i.e.*, inrolled at the outer margins. If either C' or D is recessive, the petals are flat, regardless of the dominant or recessive condition for B_1 and B_2. The dominant condition of four factors B_1, B_2, D, and H leads to the production of blue anthers. When any one of the four factors B_1, B_2, D, and H is in a homozygous recessive condition, the color is yellow.

The interaction of two of the genes that influence petal color, B_1 and D and a basic factor G for seed color conditions the development of color in the seed. When G is recessive, the color of the seed is yellow, because the yellow cotyledons are visible through the colorless seed coat. There are other factors that influence the intensity of seed color, and if G is present the seed may still be yellow. If B_1 is recessive, in the presence of G and D, there is a greenish color to the seed. When D or both B_1 and D are recessive, the color of the seed is modified from the normal brown color. Shaw *et al.* (1931) have postulated the interaction of at least seven factors that influence the inheritance of petal color in Indian varieties of flax. Their results are similar to those of Tammes. Their explanations of the inheritance of seed colors and crimping of the petals differed materially from those of Tammes. The genetic factors involved in these Indian varieties have not been studied in relation to those postulated by Tammes.

Dehiscence of the Bolls.—Three types of flax bolls may be distinguished: dehiscent, semidehiscent, and indehiscent. Most cultivated varieties of flax in the United States have the semidehiscent type of boll, where the boll opens at the apex and the five segments separate slightly along the margins. In the indehiscent type is found most of the Indian and Argentine varieties. The character is of economic importance, since the semidehiscent types thresh more easily than the indehiscent. Dillman sum-

marizes crosses made by J. C. Brinsmade, Jr., between the two types. Semidehiscent was dominant, and ratios in F_2 approached 15 semidehiscent:1 indehiscent.

Smooth vs. Ciliate Septa.—Dillman points out that in most cultivated varieties of flax the septa are ciliate, although a few varieties have smooth septa. In most American systematic botanical statements, the bolls are described as having nonciliate septa. He credits Brinsmade and A. C. Arny with having obtained a ratio in F_2 of 3 ciliated:1 smooth.

Weight of Seed and Oil Content.—Dillman has given the weight of 1000 seeds in grams for *L. angustifolium* and varieties of common flax grown in 1930 under irrigation at Bozeman, Montana. The lowest weight was that of the wild species *L. angustifolium,* at 1.5 g. per 1000 seeds, with the heaviest weight being obtained for the Lino Grande variety of 11.55 g. per 1000 seeds.

Myers (1936) studied seed weight in a cross between Redwing with a 1000-seed weight of 4.33 g., and Ottawa 770B with a mean of 5.35 g. There was a partial dominance of large seeds in F_1. One hundred F_3 lines were grown, and studies of seed weight were made in comparison with Redwing and Ottawa 770B. One F_3 line had a seed weight nearly as low as Redwing, with a relatively low variance. Two F_3 lines had as great or greater seed weight than Ottawa 770*B*, although the variance for both lines was significantly greater than that of the parents. In this cross it is apparent that weight of seed cannot be placed on a simple or definite factor basis.

Dillman found that the oil content may vary from 33 to 44 per cent or more, depending upon the interaction of heredity and environment. Johnson (1932) studied the oil content of 46 varieties of flax grown in replicated rod-row trials at University Farm, St. Paul, Minnesota, in 1929 and 1930. The material included both Argentine and domestic varieties and selections from varietal crosses. The correlations for weight of 1000 seeds and oil content were +0.72 and +0.78 for the 2 years, respectively. Dillman studied 124 varieties and strains grown at San Antonio, Texas, in 1926, that ranged in weight of 1000 seeds from 3.5 to 7.5 g. and in oil content from 36 to 44 per cent. He obtained a correlation between seed size and oil content of +0.70.

Dillman placed varieties in four groups on the basis of seed size, small, midsize, large, and very large. In general, the varieties with larger seed tend to have higher oil content and selection for seed size in a cross between parents that differ in seed-size aids in obtaining varieties with higher oil content.

Inheritance of Quality of Oil.—The drying quality of oil is dependent upon the quantity of oxygen absorbed in the process of drying to form the characteristic paint film. The chemist determines the relative drying quality of oils by the absorption of iodine per unit quantity of oil, the drying quality being expressed as the iodine number. Values may range from 150 to 200 in extreme samples. Johnson obtained a negative correlation coefficient of -0.31 for drying quality and weight of 1000 seeds, using 46 varieties grown in rod-row trials in 1930.

Arny (1936) has studied the inheritance of iodine number in several crosses between common varieties. The character is influenced rather strongly by environmental factors, individual plant determinations in the same pure line giving rather wide ranges in iodine index. Iodine index of parent plants of Bison and Ottawa 770B and of the F_1 and F_2 generations are given in Table 16.

TABLE 16.—IODINE NUMBER OF OIL FROM INDIVIDUAL PLANTS OF BISON AND OTTAWA 770B AND FROM THE F_1 AND F_2 GENERATIONS OF CROSSES BETWEEN THESE TWO VARIETIES

University Farm, 1933*

Culture	154–156	157–159	160–162	163–165	166–168	169–171	172–174	175–177	178–180	181–183	Total
Bison	3	3	7	14	6	4	..	..	.	..	37
770B	..	..	..	..	.	.	2	14	7	..	23
Bison × 770B F_1	..	..	..	4	3	5	.	..	..	..	12
Bison × 770B F_2	2	10	24	24	38	37	20	13	6	2	176

* Unpublished data kindly furnished by A. C. Arny.

In this cross, the F_1 resembled the low-iodine-index parent, although dominance was not complete. Segregation occurred in F_2. In backcrosses of the F_1 to the parent with higher iodine index, Arny obtained a 1:1 ratio when plants that fell within the range of the higher iodine-index parent were considered homozygous and those with a lower iodine index were classified as

heterozygous. These and other data led to the conclusion that a single factor pair was responsible for the main differences in quality of oil in most of the crosses studied.

Arny (1936) found rather close linkage between iodine index and seed color. The following results (cited by Dillman 1936) are from backcrosses in the coupling phase.

Cross	Year crown	Yellow seed		Brown seed	
		High	Low	High	Low
(Bison: brown, low × C. I. 355; yellow, medium) × C. I. 355....	1933	64	3	12	56
(C. I. 355 × C. I. 423; yellow, high) × C. I. 355.....	1934	25	7	6	21
(Bison × C. I. 391; yellow, high) × C. I. 391.....	1934	75	4	13	87
Total.....	....	164	14	31	164

The calculated recombination percentage of 12.0 ± 1.7 was obtained.

DISEASE RESISTANCE

Bolley, about 1900, in North Dakota, discovered the organism that causes wilt in flax and named it *Fusarium lini* Boll. He was one of the first to produce an artificial epidemic of a plant disease as an aid in selecting for resistance. His early work of selection for wilt resistance in flax and for disease resistance in other crop plants emphasized the importance and desirability of breeding for disease resistance.

Wilt Resistance.—The severity of infection with wilt is greatly influenced by environmental conditions, particularly soil temperatures, heritable differences in the degree of resistance of different varieties, and physiologic races (Broadfoot 1926) of the pathogen that causes the disease. Tisdale (1916, 1917) made important contributions to the nature and inheritance of wilt resistance. High temperature was a favorable agent in overcoming resistance. The fungus penetrates the flax plant through the stomata of seedlings, the root hairs, or through the young epidermal cells. In a resistant plant, the fungus on entering stimulates cork-wall formation of cells adjacent to those attacked.

Tisdale studied the inheritance of wilt reaction in crosses between resistant and susceptible strains. Some F_1 crosses were much more resistant than others, and in some crosses there appeared

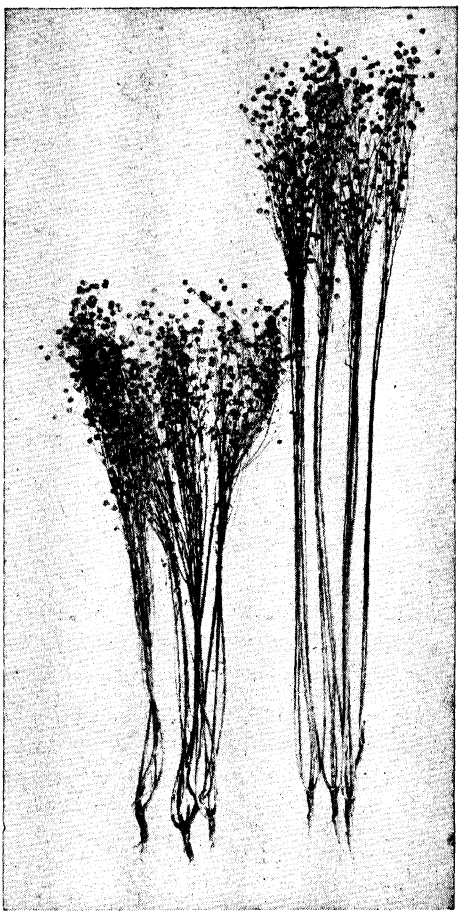


FIG. 25.—Two varieties of flax grown at St. Paul, Minnesota, showing range in height. Left: Redwing, a typical seed-flax variety. Right: Cirrus, a typical fiber flax. Varieties of fiber flax are taller and less branched than varieties of seed flax and produce lower yields of seed.

to be a dominance of resistance, whereas other crosses indicated a dominance of susceptibility. Although segregation occurred in F_2, the results could be explained only on a multiple-factor basis.

Bolley (1912) was unable to explain adequately the gradual accumulation of resistance when the crop was grown on wilt-sick

soil and the loss of resistance that was often observed after a wilt-resistant variety had been introduced. He favored the idea that disease resistance developed by a gradual accumulation of resistance under infection conditions. He was an early leader in developing wilt-resistant varieties, and the variety Bison selected by Bolley is the most widely grown wilt-resistant variety in the flaxseed area of Minnesota, North Dakota, and adjacent states. Barker (1923) made a careful study of these problems and found that some varieties contained no resistant genotypes and that in these cases resistance was not developed from a constant association with the pathogen. He found also that wilt-resistant pure lines did not lose their resistance when grown on wilt-free soil.

In a study of reaction to wilt, parental lines were self-pollinated by Burnham (1932) for at least three generations to ensure homozygosity. Studies were carried on under field conditions in wilt-infested soil, and the flax was planted late to ensure optimum infection. Certain strains of flax used as parents were completely susceptible; others, highly resistant; but occasional wilted plants were found in resistant lines used as parents. These were not believed to be genotypic variations. The extent of wilting obtained in progeny tests of different inbred parents ranged from highly resistant through partially susceptible to highly susceptible. Because of the nature of the disease, plants for producing F_2 and F_3 progenies were grown on wilt-free soil and random F_3 lines used to study the genetics of wilt reaction. From several crosses between resistant and susceptible parents, the mean for wilting of the F_2 was generally intermediate between that of the parents, and F_3 lines were obtained with mean percentages of wilting that ranged from high resistance to complete susceptibility. Approximately 1 out of 10 F_3 lines were as resistant as the resistant parent. In several crosses between resistant parents, there was a high percentage of susceptibility in F_2, indicating that the parents may have differed in factors for resistance. The difficulty of making a genetic analysis is due partially to the variability in wilt reaction from season to season and to variations in wilt reaction of the same pure line in different parts of the wilt nursery. The number and nature of genes responsible for wilt resistance could not be determined. Even though more than a single factor pair for wilt reaction was necessary to explain

the results, it appeared relatively easy, in crosses between resistant and susceptible strains, to recover lines as resistant as the resistant parent.

Resistance to Rust.—The importance of rust resistance in the seed-growing areas in North America is generally recognized. In a recent report, Flor (1940) has listed 24 physiologic races of *Melampsora lini* (Pers.) Lev. that have been differentiated on the basis of the reaction of 11 varieties of flax. No varieties of flax were found that were resistant to all races. Ottawa 770B, which has been used extensively in studies of the inheritance of rust reaction, and flaxes of the Argentine type have remained immune from all races collected in North America. The Argentine selection was susceptible to three physiologic races—19, 20, and 22—collected in South America, whereas Ottawa 770B proved susceptible only to race 22. Varieties of flax are available that are resistant to the South American races of rust but susceptible to North American races.

Henry (1930) used Ottawa 770B as one of several immune parents in crosses of immune × susceptible. Immunity was dominant in F_1. Only a single factor pair was necessary to explain the crosses between immune vs. susceptible when Ottawa 770B was used as the immune parent. When Argentine selection was used as the immune parent, segregation was on a 15:1 basis.

Myers (1937) grouped parental varieties used in studies of inheritance of rust reaction into five groups; immune, near immune, resistant, semiresistant, and susceptible, with the use of a collection of rust for the source of infection. He explained results obtained by two allelic series where L and M are duplicate factors conditioning immunity from the collection, l^n and m^n condition near immunity, l^r and m^r are duplicate factors for resistance, and l and m are the recessive alleles conditioning susceptibility. The two series of alleles then would be L, l^n, l^r, l, and M, m^n, m^r, m. The genotype of Ottawa 770B was considered to be *LL mm*.

CHAPTER XIII

METHODS OF SELECTION FOR SPECIAL CHARACTERS

It has been emphasized in preceding chapters that the breeding of improved varieties almost invariably involves selection for many characters. Some characters can be classified easily, and visual inspection will determine the desirable ones. Other characters are difficult to evaluate, and special methods must be developed to make controlled selection possible. To make the most rapid progress possible in plant breeding, it is essential that the breeder cooperate with other plant-science specialists in order that efficient methods of selection be developed. The methods used may vary with the nature of the material, the training of the investigator, and the facilities available. The plant breeder of today can well afford to make a greater use of plant physiological technics already available and to take an active leadership in the development of new technics. Illustrations will be given of certain technics that have been developed to aid in character differentiation.

QUALITY TESTS IN WHEAT

A determination of the desirability of a particular variety of wheat, for human consumption, will depend on the use to be made of it. The more important uses include bread, macaroni, pastry, crackers, and breakfast foods. Wheat especially adapted to one use may be very inferior for some other. The science of milling and baking is highly specialized, and the satisfactory evaluation of wheat quality can be made only by the cereal technologist. This emphasizes again the need of close cooperation between the plant breeder and technologists in other fields.

Among the necessary characteristics of satisfactory bread wheats is suitable baking strength. This may be defined as the inherent capabilities of a wheat or flour to produce a loaf of good volume and satisfactory crumb grain and texture, provided it is baked under conditions that preclude yeast starvation. Some

wheats require special treatment in milling or baking to bring out optimum results, making it necessary to vary fermentation time, mixing treatment, and use of "improvers" in order to obtain a complete evaluation of breadmaking characteristics. Crumb color in the bread and pigment concentration in the flour are sometimes important, and so are the dough-handling properties.

For a discussion of milling and baking methods and tests for different properties of the flour, the student may be referred to a book published by the American Association of Cereal Chemists, "Cereal Laboratory Methods" (1941).

A complete study of the milling and baking properties of a series of wheats is relatively expensive, and larger quantities of wheat are required than can be made available during the early generations in the breeding program. A number of simple, rapid methods for evaluating certain properties of flour have been developed. To be of greatest use to the plant breeder, in selection, such methods must be rapid and inexpensive and must require relatively small amounts of wheat. None of these methods alone can replace actual baking trials under commercial conditions. One such rapid test of baking strength will be mentioned.

Wheat-meal Fermentation-time Test.—The wheat-meal-fermentation-time test originated by Saunders and Humphries (1928) and developed by Pelshenke (1930, 1933) in Germany and Cutler and Worzella (1931, 1933) in America has aroused a great deal of interest as a possible means of evaluating baking strength from small amounts of seed. The method is rapid, inexpensive, and only 15 g. of wheat is needed. It is of interest to the plant breeder as a means of selection during the early generations in a breeding program when large numbers of strains need to be tested for baking quality. The test is based on the resistance of fermenting dough (made from medium finely ground whole-wheat meal) to disintegration in water. The test consists of making a ball of dough from the wheat meal, to which a standard amount of yeast has been added, placing the dough ball in distilled water in a temperature-controlled incubation chamber, and recording the time it takes for the dough ball to disintegrate. The time required for the dough balls to break will furnish a measure of baking strength, the stronger wheats requiring a longer time.

In general, it may be said that the results of the wheat-meal-fermentation-time test have been in fair agreement with baking behavior when wheats differing materially in baking strength are studied. The test has been used to good advantage, particularly with soft winter wheats, where soft wheats with a short period of dough-ball disintegration have been selected. For wheat varieties that do not differ greatly in baking strength, the meal-fermentation-time test can not be relied on to differentiate the varieties according to their reaction in standard baking trials. This appears to be true particularly with the hard wheats and in breeding experiments in which both parents have good baking strength.

COLD-RESISTANCE TESTS WITH WHEAT

Cold resistance of winter-wheat varieties is best measured in field tests in the region in which the wheat is to be grown. Differential killing is obtained only in certain years, and slight depressions in the field often lead to killing in "patches" with little relation to varieties. The development of a satisfactory laboratory test would be of great aid in selecting for cold resistance.

Numerous investigators in the hard-red-winter- and soft-red-winter-wheat regions of the United States have shown that artificially produced low temperatures could be used as an index of the ability of strains of winter wheat to survive in the field. The methods used and results obtained in a recent study by Weibel and Quisenberry (1941) will be given briefly.

Thirty varieties of winter wheat grown in the cooperative Great Plains Uniform Winterhardiness Nursery were used for the study because of the great amount of information available as to their relative winter hardiness in the field.

For the test of cold resistance, seed of the varieties was sown in flats outside during the first week in October (at Lincoln, Nebraska). Good growing conditions were maintained and the plants reached the tillering stage before going into a dormant condition for the winter. Freezing tests were made November 15, December 5, December 15, and January 15 by exposing the plants to temperature of -17 to -26°C. for 24 hr., in a mechanically controlled freezing chamber. After this freezing period the flats were transferred to a greenhouse maintained at 21°C and kept watered to allow live plants to recover. Survival

counts were made 10 days after freezing. Twelve replications were used in each of 2 years.

The interannual correlation between the survival of these 30 varieties in artificial freezing tests in each of 2 years was +.930. When the average survival, for 2 years, in controlled freezing tests was correlated with the survival of the same varieties in the field a coefficient of +.866 was obtained, a highly significant value.

These wheat varieties differed markedly in cold resistance. Extensive studies in Minnesota with strains of wheat not differing greatly in winter survival have shown very little association between winterkilling under field conditions with reaction in controlled freezing tests. Winter hardiness involves more than cold resistance. Alternate freezing and thawing, "heaving" of the soil, and other factors are of importance also.

SHATTERING IN WHEAT

Most American varieties are relatively resistant to shattering, probably because of selection for nonshattering during the breeding programs, although marked differences in this character may be observed. Shattering probably is of greater importance in the Pacific northwest than in other regions, since wheats in that region frequently are allowed to stand in the field after ripening for longer periods of time before being harvested. Wheat breeders in China have noted that one of the most undesirable characters of Chinese varieties is their extreme susceptibility to shattering, as contrasted with introduced varieties (Chang 1940).

Vogel (1938) studied the relation of the amount of mechanical tissue in the basal portion of the glumes to shattering in wheat. In general, a direct relationship was found between relative resistance to shattering and the extent of mechanical tissue at the breaking point of the glumes. Chang (1940) found a direct relationship between shattering and the amount of strengthening tissue in the inner basal portion of the empty glume and the peripheral region of the basal portion of the lemma.

A simple machine was constructed by Chang to determine resistance to shattering. This instrument was constructed in such a manner that turning a crank would cause a rubber paddle to beat the wheat heads, held on a shattering board, and thresh a portion of the grain. Three heads were tested in each trial

and 20 trials used for each plot. The mean percentage of shattering, in different varieties, obtained by the use of this instrument, varied from 1.3 to 29.6. There was good agreement between shattering under field conditions and in the controlled studies with the shattering machine.

DORMANCY IN RELATION TO BREEDING

Afterharvest sprouting may be a problem in the grain fields of many parts of the world. Varieties are known to vary greatly in length of the dormancy period after grain is ripe. In certain regions, the plant breeder wishes to select strains or varieties that are not susceptible to early germination in order to escape the losses in yield and in quality from germination of the grain in the shock as a result of rains after harvest.

Larson *et al.* (1936) studied the length of the rest period of common varieties of wheat, oats, barley, and rye by germination tests at three stages of ripeness: soft dough, hard dough, and ripe. The rest period was longest in immature seeds. The length of the rest period varied greatly with the variety. In general, winter wheats had a shorter rest period than spring wheats. The spring-wheat varieties Mindum, Marquillo, Kubanka, and Thatcher were found to have a long rest period.

Harrington and Knowles (1940*a*) presented the results of tests of the length of the dormancy period of varieties of wheat and barley. Head samples were collected from the varieties to be studied at the stage of maturity when the lower kernels on the spikes could be indented with difficulty with the thumbnail. Germination tests were made at intervals of 4, 8, 12, 16, 21, 26, and 36 days after maturity.

The varieties of spring wheat varied from the inability of Reliance to remain dormant more than 2 days after maturity to the ability of Renown to hold a high degree of dormancy for 2 weeks.

In another paper, Harrington and Knowles (1940*b*) concluded that the failure of the seeds of some varieties to germinate after exposure to moist weather for several days after harvest is due to the dormancy period of the varieties and not due to slow germination. Apex, Thatcher, and Renown were high in resistance to sprouting, whereas Garnet was very low. Chang (1940) also found afterharvest sprouting to be related definitely to

length of dormancy in hard red spring wheat, barley, and oat varieties.

Harrington and Knowles found that the variation in amount of sprouting of strains from crosses was related directly to the sprouting characteristics of the parents. Transgressive segregation occurred in some crosses, strains more resistant to sprouting than either parent being obtained. Tests of the amount of sprouting immediately after harvest can be used in selecting strains that are not deficient in this character.

LODGING IN SMALL GRAINS AND CORN

Lodging frequently results in serious losses in yield and quality. It is a difficult character to evaluate, since it is affected by numerous characters of the plant and conditions of the environment. In some seasons little or no lodging is obtained. In other seasons storms may cause most or all varieties to lodge. This situation naturally has led investigators to study differences in plant characters that might be associated with lodging.

Holbert (1924), Hall (1934), and others have found lodging in corn to be significantly correlated with the force necessary to pull the plant from the soil. This has led to determinations of the pulling resistance of inbred strains and hybrids of corn as a measure of their ability to withstand lodging.

Salmon (1931) devised an instrument for measuring the strength of straw of small grains. Strength of straw was measured in terms of the force necessary to break a given number of straws. Salmon showed that breaking strength of the straw was correlated with lodging behavior in the field. These results have been substantiated by several other investigators.

Atkins (1938) made an extensive study of strength of straw and other characters in relation to lodging in winter wheat. Straw strength was based on the force required to break five straws taken at the first upright internode above the crown of the plant. Twenty determinations were made per variety.

Relative breaking strength of straw was fairly constant from year to year, whereas lodging was not. Average lodging for several years was correlated significantly with average straw strength. In a single season, the correlation was not significant. In view of the variability in lodging from year to year, Atkins concluded that breaking strength for a single season was a more

reliable index of lodging than was a record of lodging for a single season.

Clark and Wilson (1933) correlated the average breaking strength of 30 culms per variety in spring wheat, for a single test, with the average lodging index determined from rod-row trials at four stations in Minnesota for 3 years. The correlation coefficient was nonsignificant. Breaking strength and diameter of the culms were correlated to the extent of $+.537 \pm .148$.

DROUGHT STUDIES WITH CORN

Corn yields in the Great Plains region of the United States are frequently reduced greatly by periods of very high temperature, low humidity, and deficient soil moisture in midsummer. Under these conditions, it has been noted that inbred lines and hybrids vary in their ability to withstand such periods of high temperature. Seasonal conditions vary greatly, and high temperatures do not occur every year, making selection for drought resistance difficult. Consequently a laboratory test would be highly desirable.

Hunter *et al.* (1936) and Heyne and Laude (1940), in Kansas, described a method for testing corn seedlings for resistance to high temperatures. The corn was planted in 4-in. unglazed pots, with enough seed to ensure a uniform stand of seven plants per pot. When the seedlings were from eighteen to twenty days old, they were placed in a heated room at a temperature of 130°F. and a relative humidity of from 25 to 27 per cent for 5 hr. The plants were supplied with enough water prior to the test to keep the soil moist throughout the 5-hr. test. The amount of injury, expressed as a percentage of exposed leaf and sheath tissue that had been killed, was estimated 3 days after treatment, and the number of plants killed and degree of recovery were determined 10 days after treatment.

The reaction of 90 per cent of the inbred lines of corn subjected to controlled high temperature in the seedling stage was in accord with the known behavior in the field under extreme temperatures in midsummer. All lines that were low in resistance to heat in the field reacted in a similar manner in the seedling test. The high-temperature test in the seedling stage was considered a valuable supplement to studies of drought resistance in the field.

INDUCING BIENNIAL SWEET CLOVER TO FLOWER THE FIRST YEAR

Planting sweet clover in the field results in flowering plants being obtained only in the second year. This makes for slow progress in breeding.

It has been found that extending the length of day to from 18 to 24 hr. by means of supplementary light stimulates flowering of seedlings of biennial sweet clover from 6 to 8 weeks after emergence of the seedlings. This makes it possible to grow a generation in the greenhouse during the winter months. The plants would be small, however, and selection for type would be relatively ineffective.

A seed crop the first year on relatively normal plants may be obtained by planting the seed in pots or flats in the greenhouse in February and subjecting the seedlings to enforced dormancy when they are from 2 to 3 in. tall by keeping them at 0°C. for about 20 days. After the cold treatment, the seedlings are allowed to grow under normal temperatures in the greenhouse until ready to transplant in the field. Seedlings so treated will produce fairly large plants the same season and flower profusely.

DETERMINATION OF COUMARIN CONTENT IN SWEET CLOVER

Strains and species of sweet clover, *Melilotus*, vary greatly in amount of coumarin, the compound that gives sweet clover its bitter taste and that has recently been shown by Campbell *et al.* (1940) to form the hemorrhagic substance in spoiled sweet-clover hay. Development of strains of sweet clover low in coumarin would improve the palatability of the crop greatly and reduce danger in its use as hay.

Several methods for the determination of coumarin content in sweet clover have been developed. The method developed by Clayton and Larmour (1935) and Stevenson and Clayton (1936) has been modified slightly in Minnesota and is described for leaf-tissue analysis here in detail.

Approximately 40 leaves are collected from each plant, in duplicate, well distributed among several branches. The leaves are taken from the region 4 to 12 in. from the tips of the main stem or side branches and with as short petioles as possible. It is known that the amount of coumarin varies considerably in

different parts of the plants. Consequently, care should be used in collecting the sample of leaves from the same relative portions of the plants.

The leaves collected from each plant are mixed, and a 1-g. sample for extraction and a 1-g. sample for dry-matter determination are weighed. The dry-matter sample is dried in an oven kept at 105°C. for 20 hr. and the dry weight determined.

The 1-g. sample for coumarin determination is placed in a small glass vial closed with a cork stopper, frozen as soon as possible, and kept frozen until ready for analysis. The advantage of storing the frozen samples is that many plants may be rapidly sampled, and any plants that may be discarded later on the basis of disease or plant characters have not been needlessly analyzed.

In making the analyses, the sample is ground in a mortar with 1 cc. of fine, washed sand. The ground sample is transferred to a 70-cc. test tube. The mortar and pestle are cleaned during transfer with 50 per cent methyl alcohol and the sample brought up to 51-cc. volume with 50 per cent alcohol. The sample is shaken thoroughly (in a shaking machine) for 30 min. to extract the coumarin from the ground-leaf tissue. A portion (about 25 cc.) of the extract is filtered, and 5 cc. is placed in a tightly stoppered vial. This is allowed to stand in light for 12 hr. to break down the chlorophyll.

The 5-cc. sample of the filtrate is transferred to a test tube graduated for 50 cc., and 5 cc. of 1.1 per cent Na_2CO_3 and 20 cc. of distilled water are added. The mixture is now placed in a water bath at 80°C. for 15 min., removed, cooled to room temperature; 5 cc. of ice-cold diazonium solution is added, and the volume made to 50 cc. with distilled water and mixed. The sample is then allowed to stand for 2 hr., after which the amount of coumarin is determined through comparison with standard solutions with known amounts of coumarin.

Twelve standards containing 0.0 to 1.2 mg. of pure coumarin per 50 cc. are made up. To do so, the requisite amount of standard coumarin solution is placed in a test tube graduated for 50 cc., about 20 cc. of distilled water, 5 cc. of alfalfa extract,[1] and 5 cc. of 1.1 per cent Na_2CO_3 are added. The test tubes are placed

[1] The alfalfa extract is prepared by adding 500 cc. of 50 per cent methyl alcohol to 10 g. of finely sliced alfalfa, shaking for 2 hr., and filtering.

in a water bath at 80°C. for 15 min., cooled, 5 cc. of the cold diazonium solution is added, the resultant solution made up to 50-cc. volume, mixed, and allowed to stand for 2 hr. Readings on these standards are obtained in a photoelectric colorimeter and a curve drawn.

The diazonium solution is prepared from two solutions, *A* and *B*, as follows:

Solution A.—Dissolve 3.5 g. of *p*-nitraniline in 45 cc. of 37 per cent hydrochloric acid, dilute to 500 cc. with distilled water, and filter. This solution keeps indefinitely if stoppered.

Solution B.—Dissolve 5 g. of sodium nitrite in 100 cc. of distilled water. Keep this solution in a dark bottle away from light. This solution should be renewed frequently, since it does not keep well.

Diazonium Solution.—Thoroughly chill a 100-cc. flask, solution *A* and solution *B*, on chipped ice. Pipette 3 cc. of solution *A* and 3 cc. of solution *B* into the 100-cc. flask, chill for 5 min., add 12 cc. of solution *B*, shake, chill for another 5 min., fill to the 100-cc. mark with ice-cold distilled water, mix, and place on chipped ice for 15 min. before using. If kept on ice, this solution will remain stable for at least 24 hr.

TABLE 17.—FREQUENCIES OF COUMARIN PERCENTAGES IN PARENT CHECKS AND IN F_2 OF CROSSES BETWEEN HIGH AND LOW COUMARIN SELECTIONS AND THEIR PARENTS

Parent or F_2	Number of plants with coumarin percentage of											
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40	0.50	0.60
Low parent...	11	25	2	1	..	2						
High parent..	..	..	..	..	1	..	..	1	4	10	12	1
F_2.....	17	7	12	8	7	4	1	19	21	43	35	35

The field samples are tested in the colorimeter, the reading being compared with the curve for the standards. Since a 5-cc. aliquot of the unknown sample represents 0.1 g. of the original, it follows that the colorimeter reading for a given standard solution in milligrams represents the percentage of coumarin in the sample for analysis. The amount of coumarin is calculated to a moisture-free basis. For coumarin analysis of seed, an incubation

period is necessary. An alternative micromethod of analysis has been described by Roberts and Link (1937).

Stevenson and White (1940) reported that through continuous selection in inbred lines a strain of sweet clover has been developed with only about one-tenth the amount of coumarin found in ordinary sweet clover. Stevenson and White crossed low- with high-coumarin selections and studied segregation in F_2. The results are given in Table 17.

The F_2 distribution appears to be definitely bimodal. There were 55 F_2 plants with 0.00 to 0.05 per cent coumarin and 153 with 0.10 to 0.60 per cent. The results indicate that low coumarin is inherited as a recessive in an apparently simple manner.

METHOD FOR DETERMINING HYDROCYANIC ACID CONTENT OF SINGLE PLANTS OF SUDAN GRASS

Individual plants of sudan grass vary greatly in hydrocyanic acid content. Since HCN is extremely toxic, it is highly desirable to breed strains of sudan grass free from or very low in HCN content. If single plants are to be the unit of selection, a test for HCN must be rapid and relatively inexpensive.

Hogg and Ahlgren, at Wisconsin (unpublished), have used a procedure based on a method developed by Nowoşad and MacVicar (1940). A description of this method, by Henry L. Ahlgren (unpublished communication) is given here.

The method consists of placing .15 grams of green plant material, cut into short pieces with a scissors or macerated, in a test tube, adding three or four drops of chloroform, and suspending a strip of moist filter paper saturated with sodium picrate solution above the mixture. The saturated filter paper is held in place with a cork stopper which is used to seal the test tube. The mixture is incubated at room temperature (20°C.) for 12 to 24 hours. The sodium picrate present on the filter paper is reduced in the presence of hydrocyanic acid. The color is dissolved out of the paper by placing the paper in a clean test tube containing 10 cc. of distilled water and is matched with color standards. The test is sufficiently accurate quantitatively for the selection of plants low in hydrocyanic acid. The results may be expressed in relative terms such as "high," "medium" or "low" or in approximate P.P.M. based on the percentage of dry matter in the sample.

Tillers from 5 to 7 inches in height can be used regardless of the height of growth of the remaining portions of the plant. The samples for

analysis for hydrocyanic acid are taken from that portion of the tiller immediately below the uppermost leaf collar.

The reagents and standards are prepared as follows:

The alkaline picrate solution is prepared by dissolving 25 g. of Na_2CO_3 and 5 g. of picric acid in 1000 cc. of distilled water.

Chloroform of Merck's U.S.P. grade is used.

The color standards are prepared by dissolving 0.241 g. KCN in 1000 cc. of water. This gives a stock solution containing 0.1 mg HCN per cc. Place 5 cc. of the alkaline picrate solution and 5 cc. of the KCN solution in a test tube. Add the following amounts of the KCN-alkaline picrate solution to eight test tubes

Tube Number	Cc. Solution
1	0.00
2	0.10
3	0.20
4	0.40
5	0.60
6	0.80
7	1.00
8	1.60

Bring the volume of each test tube up to 10 cc. by adding distilled water and heat to boiling in a beaker of water. Permit test tubes to stand in boiling water for five minutes. Stopper tubes and keep in a cool place. The number of milligrams of HCN present in each test tube is as follows: tube 1, 0.00; tube 2, 0.005; tube 3, 0.01; tube 4, 0.02; tube 5, 0.03; tube 6, 0.04; tube 7, 0.05; and tube 8, 0.08. These standards can be used for two weeks.

The test paper is prepared by cutting sheets of filter paper into strips 10 to 12 cm. long and 0.5 cm. wide and saturating them with alkaline picrate solution.

CHAPTER XIV

DEVELOPMENT OF METHODS OF CORN BREEDING

SELECTION WITHOUT CONTROLLED POLLINATION

Methods of corn breeding have been studied extensively since the introduction of the ear-to-row method of breeding by Hopkins (1899) in 1896. This consisted of growing and studying the progeny of each ear selected in a single row and continuing selection from the better yielding rows. Later the method was improved by replication of rows from each ear and detasseling a part of the rows from which seed was selected to prevent too close inbreeding. Williams (1905, 1907) first suggested a remnant method by which a part of each ear was saved to use for increase after the better yielding rows had been determined. By this plan three plots were needed each year, the ear-to-row trial plot, an increase plot, where the better lines as determined from the ear-to-row trial were increased, and a multiplication plot from seed produced the previous year in the increase plot. He also suggested cooperation and exchange of material among several breeders as a means of avoiding too close inbreeding. Montgomery (1909) suggested that the ear-to-row plot be used only once in several years. In intervening years, a seed plot was planted, and selection of seed was made from vigorous plants in perfect stand hills.

The usual result from this type of selection is well illustrated by studies made by Kiesselbach (1922), in which the yields given represent averages from 1911 to 1917.

Data obtained gave an opportunity to compare the yields of four different methods of seed selection and were as follows:

Type of Selection	Average Yield, Bu.
1. Original Hogue's (without selection)	53.6
2. Continuous ear-to-row since 1903	53.3
3. Increase from single high-yielding strain selected in 1906	47.7
4. Increase from composite of four high-yielding strains selected in 1906	55.0

In method 2, the better ears from the highest yielding strains were selected, whereas in method 3, the remnants of the high-yielding strain were planted in an isolated plot, and in subsequent years the better developed ears were selected. Method 4 was an increase from ear-to-row trials made in 1906 and 1907, where the four high-yielding ears gave an average yield of 79.4 bu., compared with 64.4 bu. for the original. Subsequent selection was made in the same manner as in method 3.

It is generally agreed that the ear-to-row method is valuable as a means of selection with an unadapted variety but, in general, of little use with an adapted variety. Several studies may be summarized to emphasize these conclusions.

Hayes and Alexander (1924) compared various methods of selection in isolated plots, using Rustler White Dent, which had been grown previously in central Minnesota for many years without close selection to type. The methods of selection were as follows:

1. Selection of good ears at husking.
2. Selection during seed-corn week in the first half of September, from perfect stand hills and vigorous plants, without close selection to ear type.
3. Selection as in method 2 and then reselection for ear type, *i.e.*, good buts, medium dent, straight rows, cylindrical ears, 14 to 16 rows, good ear length.
4. Montgomery's method from 100 ear-to-row plots, where remnants from the 25 higher yielding ears were bulked and subsequent selection was carried on by method 3.
5. Williams' method, F_1 cross of remnants of three better yielding ears.
6. Multiplication of seed produced by method 5.

The following data are an average of 4 years' results, except as noted.

Method of Selection	Yield, Bu.
1	54.5 ± 0.8
2	54.3 ± 0.8
3	53.2 ± 0.7
4	55.2 ± 0.8
5	55.5 ± 0.8
6	96 per cent of method 1. 3 years

Comparing methods 2 and 3, by pairing the differences for each of the 4 years, the chances were 37:1 that the difference in yield-

ing ability was significant. These data indicate a harmful result from close selection to ear type.

Smith and Brunson (1925) compared ear-to-row breeding with simple mass selection in an isolated field of several acres, making comparative trials over a 10-year period. They started originally with 990 ears in an ear-to-row trial, selecting the 40 high-yielding and 40 low-yielding, respectively. The remnants of ears used to plant the high-yielding rows were mixed to make a high-yielding group, and the remnants of the low-yielding ears were mixed to make a low-yielding group. They continued breeding plots separately for high yield and low yield, selecting 40 ears for each type of selection, respectively, in subsequent years, and detasseled alternate halves of rows, saving the seed from the detasseled half for planting next year. Four ears were selected from each of the 10 highest yielding rows in the high-yielding selections and, similarly, 4 ears from each of the 10 lowest yielding in the low-yielding selections. Each of the three methods of selection—simple mass, high, and low yield—were carried in isolated plots. Yield trials were made in another plot with the use of composite samples of seed. The chances were very great that the high selection yielded more than the low, but the odds were only approximately 3:1 that the high selection yielded more than the nonpedigree. Smith and Brunson concluded that continuous ear-to-row breeding was of little value.

It seems unnecessary here to summarize the extensive experiments that have been made to determine the relationship between ear characters and yielding ability. In general, the well-known experiments of Williams and Welton (1915) and many others show no close relationship between ear characters and yielding ability. The probable reason for these results can be appreciated by referring to a study reported by Garrison and Richey (1925) on the effects of continuous selection with Boone County White (C. I. 119). They selected continuously for 6 different ear types for an 8-year period and compared the yielding ability with unselected seed of Boone County White. Each type of selection was made in an isolated seed plot, and mixed seed from at least 50 ears was used to plant each plot. The following types of selection were made:

1. Strain 1.—Rough ears, 8 in. or more in length, with 20 or more rows of crease- to pinch-dented kernels.

2. Strain 2.—Rough ears, 8 in. or more in length, with 16 rows of crease- to pinch-dented kernels.

3. Strain 3.—Smooth ears, 10 in. or more in length, with 20 or more rows of dimple to slightly crease-dented kernels.

4. Strain 4.—Smooth ears, 10 in. or more in length, with 14 rows of dimple-dented kernels.

5. Strain 5.—Smooth ears, 10 in. or more in length, with 12 rows of dimple-dented kernels.

6. Strain 6.—Smooth ears, any length, with eight rows of dimple-dented kernels. This strain originated from a few eight-rowed ears found among those in strains 4 and 5 in 1918.

Selection was effective, since row numbers were rather rapidly modified. The following quotation taken from Garrison and Richey (1925) gives a good idea of the more important results and conclusions.

> Without regard to the reason, it is evident that close selection to any type, as practiced in these experiments, resulted in decreased productiveness. The most productive strain, No. 4, the 14-rowed smooth selection, yielded 8.4 ± 0.20 per cent less than C. I. No. 119, and the least productive, No. 3, the 20-rowed smooth selection, 14.3 ± 0.19 per cent less. The 14-rowed smooth and the 16-rowed rough selections, Nos. 4 and 2, were more productive and also departed less from the characteristic condition of the parent variety than the others.
>
> In their practical application the experiments indicate that a decrease in vigor and productiveness similar to that following inbreeding may result from too close selection for a particular kind of ear. Careful experiments have failed to demonstrate a marked consistent superiority for any specific kind of ear. Other experiments have shown that the yields of crosses between varieties of corn frequently are more productive than the average of the parents, thus indicating that the parent varieties are too homozygous to permit maximum yields. Just what constitutes too close selection is not known. In view of the lack of evidence in favor of any particular kind of ear and the abundant evidence of the decreased yields that follow close breeding, however, it seems best to stay on the safe side by avoiding such close selection.
>
> In view of the lack of evidence of marked consistent superiority for any particular kind of ear, it is unfortunate to teach that uniformity among the ears of a variety of corn is desirable by attaching importance to uniformity of sample, as is done in corn shows.

These and many other similar studies show that mass- or individual-plant methods of selection with an adapted variety

cannot be expected greatly to increase the potential yielding ability of the variety, and for this reason plant breeders have rather generally adopted controlled pollination methods of breeding during the last 15 years. The student who may wish to make a more extensive study of earlier methods of corn breeding is referred to a paper written by Richey (1922) that reviews the studies in considerable detail and contains, also, citations of the more important literature.

The conclusions to be reached from these studies is that simple mass selection for vigor of plant, time of maturity, etc., is all that is worth while and that close selection for ear type is not desirable. These conclusions have aided in a rather rapid acceptance of modern methods.

EARLY STUDIES OF SELF- AND CROSS-FERTILIZATION WITH CORN

Although Beal, in 1876, at Michigan, suggested the use of F_1 crosses between strains of corn for a commercial crop and Morrow and Gardner, in 1892, in Illinois, gave an outline of a method for producing F_1 seed and presented further data regarding its value, the utilization of hybrid vigor in corn has been developed only after extensive experiments on the effects of cross- and self-fertilization. Studies by East, at the Connecticut Experiment Station, and of Shull, at Cold Spring Harbor, have been discussed under the heading of Heterosis. Both started studies of the effects of self-pollination in corn in 1905. One of the present writers worked under East's direction in 1909 and had charge of the corn-breeding program at the Connecticut station from 1910 to 1914. Part of the self-pollinated lines started by East, in 1905, have been continued at the Connecticut Agricultural Experiment Station until the present time, being carried on by D. F. Jones since 1915. Because of the relation of principles learned with corn to the breeding of other cross-pollinated crops, it may be desirable to mention other early investigators who have studied self- and cross-fertilization with corn. G. N. Collins, of the U.S. Department of Agriculture, who was interested chiefly in fundamental principles, published his first paper on corn breeding in 1909. Vice-president Henry A. Wallace started studies of self-pollination and selection in 1913 as a private enterprise. This led eventually to the formation of the Pioneer

Hi-Bred Corn Company of Iowa. Self-pollination and selection with corn was begun at the Minnesota station in 1914. F. D. Richey, who began self-pollination of corn in 1916, was placed in charge of corn improvement for the U.S. Department of Agriculture in 1922, and his leadership was responsible to a considerable extent for the rapid development of hybrids adapted to the corn belt. Studies of selection in self-pollinated lines were started also in 1916 by C. H. Kyle and J. R. Holbert as a part of the program of the Bureau of Plant Industry. Corn improvement was placed on a cooperative basis by corn-belt experiment stations and the U.S. Department of Agriculture under the Purnell Act in 1925. A committee was appointed to formulate a program. Annual meetings in the field and laboratory furnished a medium for the exchange of ideas and materials and without doubt were responsible to a considerable extent for the rapid development in recent years of adapted hybrids for all sections of the corn belt.

A summary of conclusions drawn by Shull (1910) from his studies of self- and cross-fertilization show the detailed knowledge that was available many years ago. The following summary is quoted from Shull.

1. The progeny of every self-fertilized corn plant is of inferior size, vigor, and productiveness, as compared with the progeny of a normally cross-bred plant derived from the same source. This is true when the chosen parent is above the average conditions as well as when below it.

2. The decrease in size and vigor which accompanies self-fertilization is greatest in the first generation, and becomes less and less in each succeeding generation until a condition is reached in which there is (presumably) no more loss of vigor.

3. Self-fertilized families from a common origin differ from one another in definite hereditary morphological characters.

4. Regression of fluctuating characters has been observed to take place away from the common mean or average of the several families instead of toward it.

5. A cross between sibs within a self-fertilized family shows little or no improvement over self-fertilization in the same family.

6. A cross between plants belonging to two self-fertilized families results in a progeny of as great vigor, size, and productiveness, as are possessed by families which had never been self-fertilized.

7. The reciprocal crosses between two distinct self-fertilized families are equal, and possess the characters of the original corn with which the experiments were started.

8. The F_1 from a combination of plants belonging to certain self-fertilized families produces a yield superior to that of the original cross-bred stock.

9. The yield and the quality of the crop produced are functions of the particular combination of self-fertilized parental types, and these qualities remain the same whenever the cross is repeated.

10. The F_1 hybrids are no more variable than the pure strains which enter into them.

11. The F_2 shows much greater variation than the F_1.

12. The yield per acre of the F_2 is less than that of the F_1.

Effects of self-fertilization were discussed in somewhat greater detail by East and Hayes (1912). The following statements summarize the more important results of inbreeding and selection.

1. Loss of vegetative vigor has followed continued self-pollination in all inbred lines of corn.

2. Inbred lines exhibit differences in many normal characters; for example, some inbred lines have long ears; others, short ears.

3. Some inbred lines are much more vigorous than others, even though they do not differ in degree of homozygosity.

4. Some pure strains are so lacking in vegetative vigor that they cannot be propagated.

5. Continued inbreeding leads to purity of type.

Shull (1909) outlined a pure-line method of corn breeding based on the isolation of self-pollinated lines and the use of F_1 crosses between them for the commercial crop. The difficulty of this method was the cost of seed from single crosses.

The work of Jones, at the Connecticut Experiment Station, since 1915 was continued with some of the inbred lines available from the early work of East and Hayes. The Mendelian explanation of hybrid vigor, given by Jones (1917) was of great value in an understanding of corn-breeding principles. The double-cross plan of corn breeding, developed by Jones about 1917, has helped materially to make hybrid seed production economically feasible.

CONTROLLED POLLINATION METHODS

Many workers have taken part in the extensive studies that have led to a partial standardization of modern methods of corn breeding. Some of the investigations will be briefly summarized. Although individual research has been the basis of a standardization of technic, the rather rapid acceptance of methods has been

brought about by cooperation and free exchange of ideas and material among investigators.

The following brief summaries of investigations are given to furnish a background for an understanding of the development of methods of corn breeding.

Two major problems are faced by the corn breeder. There are (1) the isolation of the most desirable inbred lines and (2) the use of these lines to produce hybrids with high yielding ability that excel also in other characters.

One of the problems not entirely solved yet and one that perhaps never will be solved is the extent of homozygosity necessary in inbred lines. Richey and Mayer (1925) made a comparison of crosses between inbred lines after 3 and 5 years of self-fertilization and concluded that there was no general advantage in yielding ability from crosses between lines inbred for 5 years over crosses between lines inbred for 3 years. They found little or no relationship between the productiveness of selfed lines and that of their crosses. Richey (1924) and Richey and Mayer (1925) found that certain lines behaved rather uniformly in different crosses and that, on the average, certain strains gave high-yielding crosses when combined with a random series of other inbred lines; *i.e.*, certain lines were good combiners.

At Minnesota, Nilsson-Leissner (1927) and Jorgenson and Brewbaker (1927) compared the yielding ability of inbred lines and of possible F_1 crosses between them by means of the correlation coefficient. The number of lines, source of material, place of study, and the correlation coefficients are given in the following summary:

Source of material	Number of inbred lines	Place	Correlation coefficient
Flints.....	9	University Farm, St. Paul, Minn.	$+.74 \pm .04$
Dents.....	13	University Farm, St. Paul, Minn.	$+.19 \pm .06$
Silver King....	10	Waseca, Minn.	$+.50 \pm .08$

Multiple correlation coefficients were calculated also between five characters of the inbred lines and the yield of their F_1 crosses

as follows: University Farm, Dents, $R = .67$; Flints, $R = .82$; Waseca, Silver King, $R = .61$.

In these studies, some lines tended to give good yields in crosses, and others generally were low combiners. Although some high-yielding inbred lines did not combine well in general, the more vigorous inbred lines, on an average, were better combiners than the less vigorous inbred lines.

Jenkins (1929) made a more detailed and extensive study of a similar nature. In a study of the relationship of the yielding ability of inbred lines and their F_1 crosses, he found, on an average, less association than in the Minnesota studies. Coefficients of correlation in 1926 and 1927 for the mean yield of inbred lines and their F_1 crosses were $+.20 \pm .03$ in both years. When the mean of several crosses was used as a criterion of the combining ability of the inbred lines and correlated with the yield of the inbred lines, coefficients of $+.32 \pm .07$ and $+.12 \pm .09$ were obtained in 1926 and 1927, respectively. When the mean yield of the cross-bred progeny was correlated with four characters of the inbred lines, indicating plant vigor and size, a multiple correlation coefficient of $+.42 \pm .05$ was obtained.

Kiesselbach (1930) has shown that advanced-generation crosses produced, on an average, as high yields in double crosses as when F_1 crosses are used as parents for the double cross. An advanced generation is the product of normal uncontrolled pollination in the progeny of an F_1 or later generation cross grown in an isolated plot. One difficulty of using advanced-generation crosses for producing double-crossed seed, as pointed out by Kiesselbach, is the reduction in seed production of F_2 and F_3 in comparison with F_1. His F_2 and F_3 generations averaged about 67 per cent as much grain yield as the F_1. Richey *et al.* (1934) found that the F_2 of 10 double crosses yielded 5 to 24 per cent less (average 15.2) than the F_1. Neal (1935) obtained an average yield in F_2 and F_3 for advanced-generation single crosses of 70.5 and 75.7 per cent of the F_1, respectively. Various investigators have suggested that advanced-generation seed be used as the male parent in the commercial crossing plot.

After obtaining as desirable inbred lines as possible, the problem remains of how to use these inbred lines in hybrid seed production. Several methods have been put in practical use. These include:

1. Single crosses.
2. Inbred-variety crosses, sometimes called top-crosses.
3. Three-way crosses.
4. Double crosses.
5. Multiple crosses.

Single crosses can be used only when the inbred lines yield sufficiently well to make seed production economical. The higher yielding inbred line should be used as the female parent.

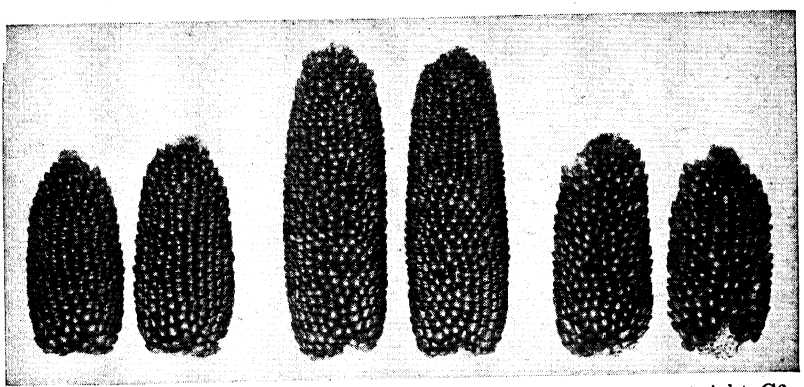


FIG. 26.—At left, C1, an inbred line of Japanese hull-less popcorn; at right, C6, another inbred; in center, the F_1 cross, Minhybrid 250. The inbreds yield sufficiently well so that the use of F_1 crossed seed for commercial planting is feasible. The F_1 cross yields approximately 16 per cent more than Japanese hull-less and has approximately 29 per cent greater popping expansion.

Single crosses are being used in producing sweet corn for canning, where uniformity of maturity and for ear characters are of major importance. If the inbred lines are reduced only to practical homozygosity by 3 or 4 years of inbreeding, single crosses in sweet corn are economically feasible. An illustration of inbred lines and the commercial F_1 cross of Japanese hull-less popcorn is given in Fig. 26.

Top-crosses, or inbred-variety crosses, may be expected to yield somewhat less than single, three-way, or double crosses, on an average. They have been used extensively in sweet-corn breeding because of their practical features. Before the best single, three-way, or double crosses have been determined, it frequently may be of value to use inbred-variety crosses, since such crosses, when carefully selected, may have a higher yield and greater uniformity than standard varieties.

In a three-way cross, a good pollen-producing inbred line is used as the male parent, and a single cross is used as the female parent. Both the inbreds used in the single cross should combine well with the pollen parent. An illustration of the parent inbred lines, F_1 cross and three-way cross, Minhybrid 301 is given in Fig. 27.

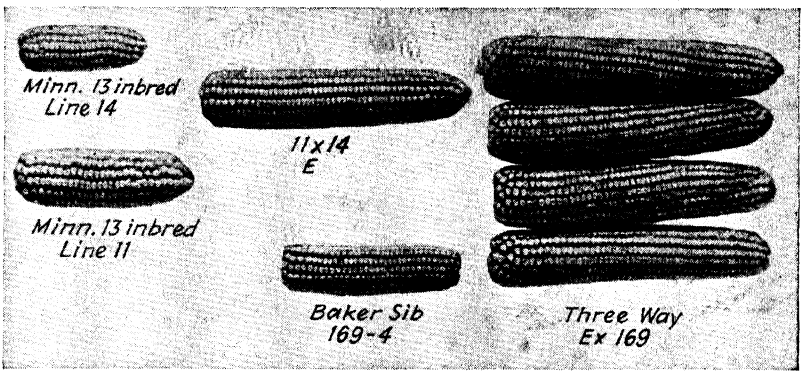


FIG. 27.—The three-way cross at the right, Minhybrid 301, produced by crossing the F_1 cross, upper center, of two inbred lines of Minn. 13 with an inbred line of Reid's Yellow Dent, lower center. At the left are representative ears of inbred lines 14 and 11 of Minn. 13.

In a double cross, two single crosses are used as the parents. Advanced-generation single crosses can be used to advantage as the male parents. This is valuable as a means of extending the pollen-shedding period of the male parent.

One of the major problems of the corn breeder is to devise some method of selecting the better crosses. Richey and coworkers advocated a series of inbred testers as a means of selecting inbred lines that were good combiners, determining the combining ability of each inbred line by crossing it with each of the testers separately. Jenkins and Brunson (1932) have compared different methods of testing the combining ability of inbred lines. In one case they compared two methods, using 37 inbred lines. The combining ability was tested in a series of crosses of each of the 37 lines with 9 tester inbred lines in 1927 and in an inbred-variety cross in 1929. The combining ability of the 37 lines determined by yields obtained by these two methods was correlated, obtaining a correlation coefficient of +.53, where .32 represented a significant relationship based on odds of 19:1.

In a second study, they used 12 early inbreds in a series of crosses with 9 comparable inbred lines and 17 late inbreds in a series of crosses with 10 comparable inbred lines, correlating the average combining ability of each line with its combining ability in top- or inbred-variety crosses. The results were as follows:

Correlation coefficient	Lines used	
	12 early	17 late
Calculated r	+.80	+.65
Significant r	.58	.48

In a third group, the combining ability in top-crosses and in a series of 10 comparable single crosses was determined with the following results:

Correlation coefficient	Lines used			
	10 early white	17 early yellow	10 late yellow	10 Lancaster
Calculated r	+ 90	+.86	+.63	+.90
Significant r	.60	.48	.63	.63

In a fourth group, 60 inbred lines of Pride of Saline were placed in 6 groups of 10 lines each, and all possible crosses were made between each line in the odd group with each 10 in the next higher even group. They determined the combining ability of each line also by the top-cross method and compared the results of the two trials, *i.e.*, combining ability in top-crosses with the combining ability obtained from an average of single crosses, for each of the 60 lines, obtaining an r of +.56 where a significant r was .26.

To determine whether crosses with a series of inbred lines used as testers are more satisfactory as a measure of combining ability, they studied the combining ability of inbred lines in two different series of inbred crosses. They used the following groups: (1) 9 early white endosperm inbreds, (2) 9 early yellow, and (3) 10 late yellow lines. In test *A*, the combining ability of each line was

determined from crosses with every other line in the same subgroup. In test *B*, the following studies were made of combining ability. The 9 early white lines were crossed with 13 other inbred lines of white endosperm; the 9 early lines of yellow were each crossed with 12 other inbred lines of yellow endosperm, and each of the 10 late lines were crossed with 17 other late inbreds. Correlating the combining ability in tests *A* and *B* gave the following:

Correlation coefficient	Groups		
	9 early white	9 early yellow	10 late
Calculated r	+.82	+.69	+.65
Significant r	.67	.67	.63

These coefficients are about the same magnitude as those obtained by correlating the yielding ability in top-crosses with that of a series of inbreds used as testers.

The results justify the use of inbred-variety crosses to determine the combining ability of inbred lines.

Johnson and Hayes (1936) studied the combining ability of 11 inbred lines of Golden Bantam in all possible single crosses and in top-crosses. The correlation between the average yield of the 11 lines in all possible single crosses and the average yield in top-crosses with Golden Bantam and Del Maiz was $+.78 \pm .12$. The combining ability of 39 lines in two series of top-crosses was studied also. Johnson and Hayes suggested the need for many replications and of making yield trials at several locations, preferably, to determine accurately in a single year the combining ability of inbred lines by the top-cross method. This plan has been adopted as a standard practice in the Minnesota corn-breeding program.

After selecting the more desirable inbred lines by the top-cross method, it is necessary to determine the best combination of lines for single, three-way, and double crosses. An actual field trial must be used to determine the value of a particular combination, but methods of prediction based on a previous knowledge of combining ability may be of value.

For a three-way cross between lines, a good pollen producer must be used as the male parent. If inbred lines 1, 2, and 3 are used in a three-way cross and inbred line 3 seems most satisfactory as a pollen parent, it is then necessary that single crosses 1 × 3 by 2 × 3 both be good producers in order that the three-way cross (1 × 2) × 3 be desirable. Thus, a three-way cross may be

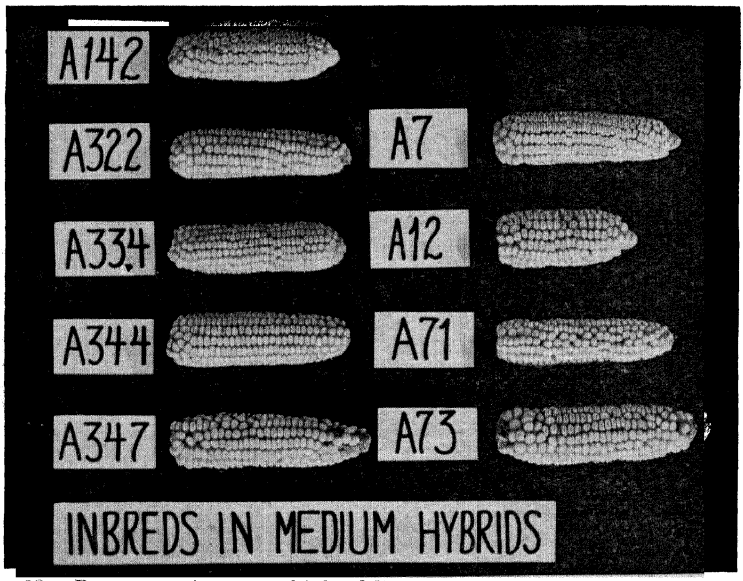


FIG. 28.—Representative ears of inbred lines used in double crosses adapted to south central Minnesota, ⅛ normal.

selected on the basis of single crosses and a knowledge of the desirability of the lines as pollen parents.

In a double cross of four lines, *i.e.*, (1 × 2) × (3 × 4), where single crosses 1 × 2 and 3 × 4 are planted alternately in a plot for hybrid seed production, it would seem desirable to use the most satisfactory cross 1 × 2 or 3 × 4 as the female parent, giving consideration to yielding ability and size and uniformity of the seed of the two F_1 crosses. Inbred lines used in double crosses adapted to south central Minnesota, the two F_1 crosses used as parents, and the double cross, Minhybrid 502, are given in Figs. 28 and 29.

Some studies have been made that furnish a logical basis for the prediction of the performance of double crosses and that aid in selecting the more promising combinations for yield trials.

Jenkins (1934) has presented a study in which 11 inbred lines were used. From these 11 inbred lines, all but 2 of the possible single-cross combinations were obtained, and the combining ability of each of the lines was also tested in an inbred-variety cross. Forty-two of the possible double crosses of these inbred lines were studied also, and four methods of predicting the

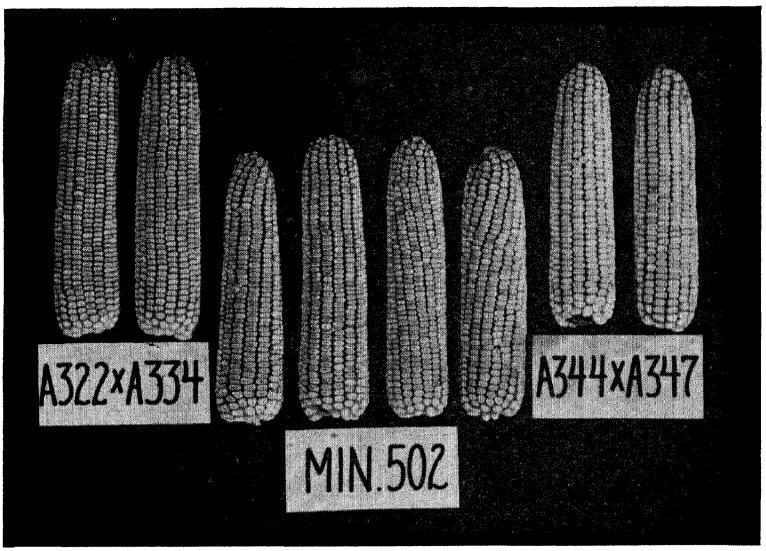


FIG. 29.—Representative ears of single crosses and of the double cross, Minhybrid 502, adapted to south central Minnesota, ⅛ normal.

probable value of these 42 double crosses were compared. These methods are as follows:

1. The yield of all six single crosses from the four lines used in each double cross was used as a basis for prediction of the probable value of the double cross. If the lines are 1, 2, 3, and 4, these single crosses are 1 × 2, 1 × 3, 1 × 4, 2 × 3, 2 × 4, and 3 × 4.

2. The yield of four single crosses, excluding the two used as parents, was used to estimate the probable performance of the double cross. If the double cross was (1 × 2)(3 × 4), the four single crosses selected to predict yielding ability would be 1 × 3, 1 × 4, 2 × 3, and 2 × 4.

3. The mean yielding ability of each line in all possible single crosses was first determined, and from these means the combining ability in a particular double cross was estimated. In double cross (1 × 2)(3 × 4), the probable value of the double cross was

estimated by averaging the combining ability of lines 1, 2, 3, and 4 in all possible combinations with the other inbred lines. By this method, the prediction value for four lines in a double cross would be the same for $(1 \times 2)(3 \times 4)$ as for $(1 \times 3)(2 \times 4)$ or other combinations of single crosses of these four lines.

4. The combining ability was determined of the four lines in each double cross by an average of their yields in inbred-variety crosses.

The value of these four methods of prediction was determined by correlating yields obtained from each of the prediction methods with the yield of 42 double crosses, with the following result:

Predicted yield with actual yield of	Correlation coefficients by methods				
	A	*B*	*C*	*D*	Significant
42 double crosses. . . .	+ 75	+.76	+ 73	+.61	39

Although prediction of the value of the double cross by method *D*, the inbred-variety cross plan, gave a lower value of *r* than methods *A*, *B*, and *C*, it is possible that this may have been due to insufficient replication in *D* or to a chance deviation rather than to the fact that this method is the least desirable or reliable of any of the four methods tested.

In discussing method *B*, Jenkins stated, "In any double cross the genes of each of the four parental lines are united only with allelomorphs of the two lines which entered the double cross from the opposite parent." Extensive studies at Minnesota have shown that this method can be used to predict the yield of a double cross about as accurately as by testing the actual double cross. Doxtator and Johnson (1936) compared the yielding value of inbred lines in double crosses on the basis of the way that they are combined and have proved clearly that it is of extreme importance how these lines are combined, *i.e.*, which combinations of single crosses are used as parents for the double cross. In general, other things being equal, the two lower yielding single crosses of the possible six should be selected as parents of the double cross.

The results of these studies are summarized in Table 18.

The predicted yields were obtained by averaging the actual yields from single crosses. Thus, the predicted yield of (62 × 67) × (66 × 68) was obtained by averaging the yields of the single crosses (62 × 66), (62 × 68), (67 × 66), and (67 × 68), which were 58.8, 17.1, 58.9, and 38.8, respectively. This gave a predicted average yield of 43.4 bu. In general, the agreement between predicted yields and actual yields obtained was very close in these studies.

TABLE 18.—ACTUAL AND PREDICTED YIELDS OF DOUBLE AND THREE-WAY CROSSES

Hybrid	Yield, bu. per acre	
	Obtained	Predicted
Waseca branch station:		
(11 × 14) × (374 × 375)	78.70	85.55
(11 × 374) × (14 × 375)	66 33	70.79
(11 × 375) × (14 × 374)	70.58	69.31
(11 × 14) × 374	81 67	86 92
(11 × 14) × 375	82.63	84.17
University Farm:		
(62 × 67) × (66 × 68)	48 4	43.4
(62 × 66) × (67 × 68)	41.8	41.7
(64 × 66) × (62 × 68)	54.1	47.5
(64 × 68) × (62 × 66)	44 5	39.8

Data from Anderson (1938) are presented to give further information regarding actual and predicted yields. The results in Table 19 give yields of single crosses in bushels per acre and show the method of predicting the yield of an actual double cross.

A comparison of actual and predicted yields of double crosses by Anderson (1938) is given in Table 20 to show the value of the method.

During recent years extensive unpublished data at Minnesota show the accuracy of predicting the yielding ability of double crosses and lead to the conclusion that such predicted yields can be used as actual measures of the yielding ability of new double crosses. By comparing the single crosses used in the predictions with standard double crosses of known yielding ability, one can, with a high degree of accuracy, accept the predicted yield and use

TABLE 19.—METHOD OF PREDICTING YIELDS OF THE THREE DIFFERENT DOUBLE CROSSES THAT CAN BE MADE FROM FOUR INBRED LINES WITH THE USE OF THE YIELDS IN BUSHELS PER ACRE OF ALL SIX POSSIBLE SINGLE CROSSES

(23 × 24) × (26 × 27)		(23 × 26) × (24 × 27)		(23 × 27) × (24 × 26)	
Single cross	Yield, bu.	Single cross	Yield, bu.	Single cross	Yield, bu.
(23 × 26)	62.6	(23 × 24)	41 7	(23 × 24)	41.7
(23 × 27)	70.8	(23 × 27)	70 8	(23 × 26)	62.6
(24 × 26)	65.6	(26 × 24)	65 6	(27 × 24)	72.1
(24 × 27)	72 1	(26 × 27)	64.2	(27 × 26)	64 2
Average	67.8	Average	60 6	Average	60.2

TABLE 20.—A COMPARISON OF ACTUAL YIELDS OF SIX DOUBLE CROSSES WITH PREDICTED YIELDS OBTAINED BY AVERAGING THE YIELDS OF THE FOUR SINGLE CROSSES NOT USED IN MAKING THE DOUBLE CROSS

Lines combined and double cross	Bu. per acre	
	Actual	Predicted
23, 24, 26, 27:		
(23 × 24) × (26 × 27)	68.8	67.8
(23 × 26) × (24 × 27)	62 4	60.6
(23 × 27) × (24 × 26)	62.0	60.2
23, 24, 26, 28:		
(23 × 24) × (26 × 28)	65.0	65 5
(23 × 26) × (24 × 28)	59.8	58.0
(23 × 28) × (24 × 26)	56.0	58.5
23, 24, 27, 28:		
(23 × 24) × (27 × 28)	71 1	69 2
(23 × 27) × (24 × 28)	58.1	59.4
(23 × 28) × (24 × 27)	58.0	60.4
Difference for significance at 5 per cent level	5.3	3.4

it in the same manner to determine the value of a double cross as if the actual yield of the double cross had been obtained. The value of the method is emphasized by giving formulas that show how many single and double crosses can be produced from n inbred lines.

The number of single, double, and other crosses that can be made from n inbreds may be calculated from the number of combinations of n inbreds taken r at a time, where r is the number of inbreds in the cross. This is given by $\frac{n!}{r!(n-r)!}$.

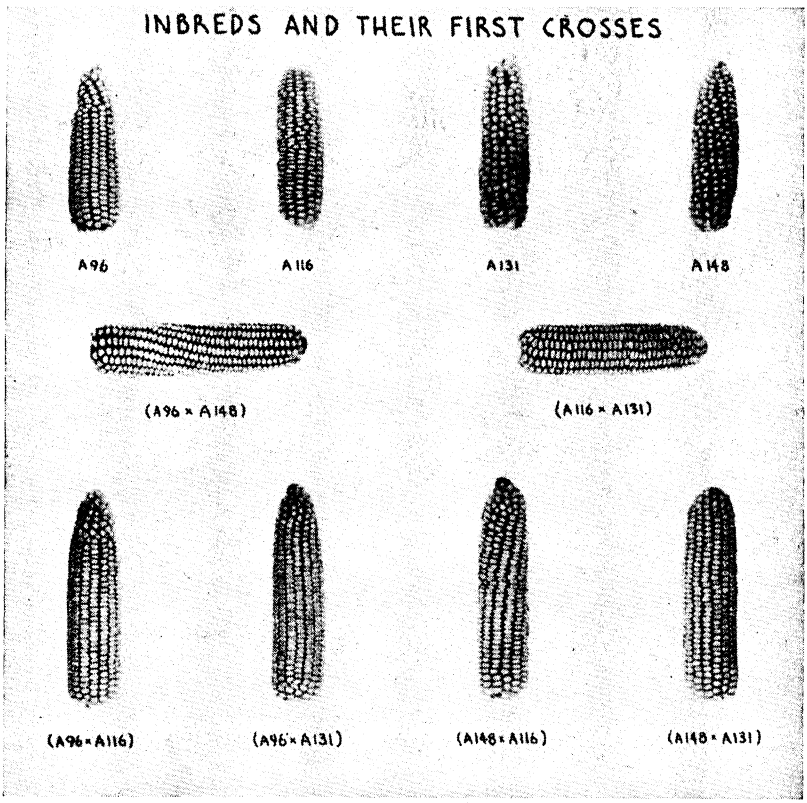


FIG. 30.—Above, representative ears of four inbred lines; center, the two single crosses used as parents to produce the double cross; below, representative ears of four single crosses used to predict double-cross yields. The double cross, produced by crossing the single crosses given in the center of the picture, matures satisfactorily in the corn-growing regions of the Minnesota Red River Valley and has good agronomic characteristics for this region.

For single crosses, $r = 2$, and the number of possible single crosses will be $\frac{n(n-1)}{2}$.

For double crosses, $r = 4$, and the formula becomes three times $\frac{n!}{4!(n-4)!}$, since three double crosses can be made from

any four inbreds. This formula may be expressed as

$$\frac{3n(n-1)(n-2)(n-3)}{24}$$

Thus, for 20 inbred lines, 190 different single crosses can be made, and from the yield trials with these one can predict the actual yielding ability of 14,535 double crosses.

BREEDING IMPROVED INBRED LINES

A problem of great importance to the breeder is the development of improved inbred lines. The following methods are available at present:

1. Inbreeding and selection from commercial varieties.
2. Inbreeding and selection from high-yielding crosses.
3. Breeding improved lines by a definite plan of crosses and selection.
   a. Pedigree methods, as with small grains.
   b. Backcrossing.
   c. Convergent improvement.

It seems unnecessary to describe these methods in great detail. Inbreeding and selection from commercial varieties has been the common practice and is necessary as the first step. As with other plant-breeding studies, it is important to use as large numbers as possible and to determine the breeding value of a line by progeny trials. A plan in common use is to grow a short row of 30 or more plants from each desirable inbred ear from the previous generation and to continue inbreeding and selection until practical homozygosity has been reached. Jones and Singleton (1935) have suggested growing only a few plants from each inbred ear, thus making it possible to grow several thousand inbred lines per acre, with the view that the more important differences will be those between lines rather than selection within lines. Jenkins (1935) studied the combining ability in top-crosses with Krug of 14 inbred lines of Iodent and 14 of Lancaster for 8 inbred generations. It was shown that the inbred lines established their individuality as combiners early and maintained it during successive generations of selfing. It was suggested that the selection of desirable combining lines should be determined by crosses made during early generations of selfing.

As with other methods of plant breeding, it is important to know the characters desired and carry on breeding studies with a

definite plan. If these characters are present in the inbred lines used in various types of crosses, then these crosses may be a promising basis as material for developing a new series of inbred lines. In some cases, this will lead to the pedigree method of breeding that has been outlined already for self-fertilized crops.

THE PEDIGREE METHOD OF SELECTION IN THE SEGREGATING GENERATIONS AFTER CROSSING INBREDS

Such a method has been used extensively at Minnesota, and its value has been discussed by Hayes and Johnson (1939). Most inbreds obtained from varieties adapted to Minnesota lacked ability to withstand lodging, and at least one inbred parent of the single crosses used as a basis for selection of inbred lines was outstanding in ability to withstand lodging. Selection was made during the segregating generations from selfed progenies for ability to withstand lodging, for smut resistance, and for other desirable characters. Many inbred lines were isolated that excelled in standing ability and in other characters. The inbred lines obtained in this way, together with the inbred parents used in single crosses, were tested for combining ability by crossing with Minn. 13 and making the necessary yield trials. When both inbred parents of a single cross were high in combining ability, as determined from yield trials of inbred-variety crosses, then practically all inbreds isolated from this particular cross also showed high combining ability. Conversely, inbreds selected from a single cross between two lines of low combining ability were mostly of low combining ability when tested in inbred-variety crosses, whereas inbreds selected from a cross of a low combiner inbred with a high combiner gave a range in combining ability from low to high. These data show that combining ability is an inherited character. Twelve characters that for the most part represent vigor of growth, such as leaf area, height of plant, volume of root clump, pulling resistance, etc., of 110 inbred lines were correlated with each other and with the yield of inbred-variety crosses. The multiple correlation between inbred-variety yields in bushels per acre and these 12 characters of the inbreds was .67, indicating that approximately 45 per cent of the variability in yield of the inbred-variety crosses was dependent upon the characters of the inbreds, leaving 55 per cent unaccounted for.

These results show the desirability of selecting vigorous inbreds, not only because of their value in seed production but also in relation to the yielding ability of double crosses. It is evident that by selecting inbred lines that have high combining ability and making crosses between inbreds that have complementary characters, followed by selection in self-pollinated lines, improved inbreds can be obtained that excel both in their inherent characters and as parents of double crosses.

GENETIC DIVERSITY

Studies by Wu (1939) and Hayes and Johnson (1939) and Johnson and Hayes (1940) of the F_1 crosses between these same inbred lines have shown the value, in relation to yield of grain, of genetic diversity of inbred lines used in double crosses. Three groups of lines based on relationship were studied, and the yields of single crosses were compared on the basis of origin. The three groups may be illustrated as follows:

Original Cross	Inbred Cultures after Selection in Self-pollinated Lines
A48 × H*	A94, A96
A9 × A26	A102, A111, A116, A122, A124
A9 × A39	A99
A39 × A26	A136, A143, A145

* A48 was an inbred from Northwestern Dent, H from Reid's, A26 from Osterland's Dent, A39 from Rustler Dent, and A9 from Minn. 13.

Group I, no parents in common; *i.e.*, A94 × A102, etc.
Group II, one parent in common; *i.e.*, A102 × A99, etc.
Group III, both parents in common; *i.e.*, A102 × A111, etc.

As would be expected, group III of single crosses yielded much less, on the average, than group I or group II, and group I of single crosses were considerably higher yielding, on the average, than group II.

Genetic diversity may be of as great value or of greater value than combining ability. This is indicated by studies of Johnson and Hayes (1940), who used inbred lines produced by the pedigree method and made crosses only between inbred lines of different genetic origin. These inbreds were classified into four groups on the basis of their yields in top-crosses, in percentage of a standard group of hybrids and Minn. 13 that was used as the variety parent of the inbred-variety crosses. Four yield classes

in percentage values were 80 to 89, 90 to 99, 100 to 109, and 110 or above. In the final classification, hybrids in 80 to 99 per cent classes were considered as low combiners and 100 or above as high combiners. The inbred lines were then studied in single crosses in three groups of crosses: high × high, low × high, and low × low. The single crosses were then placed in frequency distributions in comparison with recommended double crosses of like maturity. Results are given in Table 21.

TABLE 21.—SUMMARY OF FREQUENCY DISTRIBUTION OF SINGLE-CROSS YIELDS AT THREE LOCATIONS WITH THREE REPLICATIONS AT EACH LOCATION, WHEN COMPARED WITH RECOMMENDED DOUBLE CROSSES OF SIMILAR MATURITY IN RELATION TO THE COMBINING ABILITY OF THEIR INBRED PARENTS

Type of cross	Class centers of plus and minus 1 to 8 times the standard error of a difference									Total	Mean class
	−7 to −8	−5 to −6	−3 to −4	−1 to −2	0	+1 to +2	+3 to +4	+5 to +6	+7 to +8		
Low × low	..	1	1	2	4	4	..	..	..	12	−0.50 ± 0.66
Low × high	1	3	..	11	6	16	9	5	1	52	+1.06 ± 0.42
High × high	..	1	5	12	8	33	20	4	..	83	+1.10 ± 0.24

Low × low combiners yielded distinctly less in single crosses than low × high or high × high combiners. However, low × high and high × high yielded about the same, on the average, when the inbred lines used in the study were genetically of rather diverse origin. These results emphasize the value of genetic diversity of inbred lines used in hybrid combination.

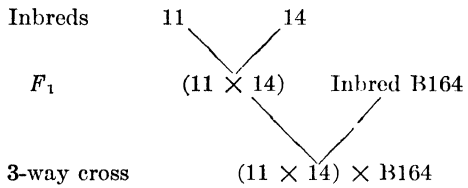
Eckhardt and Bryan (1940), at Iowa, have given data from a series of double crosses that also emphasize the value of genetic diversity from the standpoint of origin in relation to the yielding ability of double crosses. If inbreds from one variety were designated as A and B and from the other as X and Y, they compared the yields of the double cross $(A \times B)(X \times Y)$ with $(A \times X)(B \times Y)$ or $(A \times Y)(B \times X)$. The double crosses illustrated by $(A \times B)(X \times Y)$ were decidedly superior to the combination from the same inbreds $(A \times X)(B \times Y)$. These facts show that crosses between inbreds that originated from

different varieties may be expected to be superior, on the average, to comparable crosses from inbreds originating from the same variety.

THE BACKCROSS

Backcross methods may be desirable in some cases to add one or two characters to an available inbred line.

An undesirable character of the three-way cross Minhybrid 301 is smut susceptibility, dependent to a large extent on the extreme susceptibility of inbred B164, which is used as the male parent. The pedigree of the three-way cross is as follows:



B164 has been improved in its smut susceptibility by backcrossing, as illustrated in the following outline:

B164, Smut Susceptible — Early Inbred C37, Smut Resistant

Method:

1. (B164 × C37) × B164.
2. [(B164 × C37) × B164] × B164.
   Selection in 1 and 2 for smut-resistant plants to backcross to B164.
3. Self-pollination and selection for smut resistance for 3 years.

In field trials in 1938 and 1939 at the Waseca branch station, several inbred lines were obtained after self-pollination and selection had been practiced for 2 and 3 years, that during both years had less than 10 per cent of their progeny smutted, whereas B164 in near-by rows ranged from 85 to 90 per cent of its plants smutted.

CONVERGENT IMPROVEMENT

This method of breeding suggested by Rickey (1927) and discussed by Richey and Sprague (1931) has been used rather extensively in recent years.

The value of the method, which is equivalent to double backcrossing, is that it furnishes a plan for the improvement of each of two inbred lines that combine well in a single cross without

modifying the yielding ability of the single cross. Richey and Sprague have stated that the theoretical basis for convergent improvement assumes that:

1. Selfed lines that give a high yield in F_1 carry together important dominant genes necessary for yield and are alike in necessary recessive genes.

2. Excess yield of the F_1 cross over one parent is attributable to favorable dominant genes received from the other parent.

3. Back-pollinating a cross as $N \times R$ to R, in several successive generations, without selection and in the absence of linkage, will recover the genotype of the recurrent parent R, according to the series $\frac{1}{2}$, $\frac{3}{4}$, $\frac{7}{8}$, etc.

4. Selection of the more vigorous heterozygous crosses during the period of back-pollination will retain some of the dominant favorable characters of N in a heterozygous condition.

5. Selection within selfed lines after back-pollination will produce a line homozygous for R and for some dominant favorable N genes.

6. Recovered lines $N(R')$ and $R(N')$ will differ in fewer dominant favorable genes than N and R. Repetition of backcrosses would gradually produce better and better lines with more and more favorable genes in a single strain.

The more important steps in the convergent improvement program consist of the following:

1. Selection of a high-yielding desirable F_1 cross.

2. Backcrossing the F_1 to both parents and then further backcrossing in successive generations in two series to the respective parents.

3. During the period of backcrossing, selection of vigorous plants that have other desirable characters and use of these in making the backcrosses.

4. Selection within selfed lines after several generations of backcrossing.

5. Repetition of the four steps with the better recovered inbred lines in order to obtain further improvement.

The practical possibilities from backcrosses have been studied experimentally by Richey and Sprague (1931) and compared with the theoretical as a means of learning whether heterosis is dependent upon the interaction of dominant factors, a part of which are contributed by each parent. In this summary, R_1, R_2,

R_3, etc., refer to the first, second, and third backcross progeny, respectively. The results are summarized for six crosses. The theoretical expectation is calculated by subtracting the yield of the inbred R parent from the F_1 and assuming that one-half of this difference will be retained in the first backcross generation, one-fourth in the second backcross, etc., if selection is not practiced. The amount of vigor retained if selection is practiced will represent the value of this selection. The following results were obtained:

Cross or selfed line	Actual yields	Theoretical
$(R \times N)F_1$	19.7 ± 0 7	
$(R \times N)R_1$	11 7 ± 0.5	11.7
$(R \times N)R_2$	8 2 ± 0.4	7.6
$(R \times N)R_3$	7.2 ± 0 3	5.6
$(R \times N)R_4$	5.8 ± 0.3	4.6
$(R \times N)R_5$ (only 2 crosses)	4.5 ± 0.2	4.1
$(R \times N)R_6$ (only 1 cross)	4 6 ± 0.3	
R selfed	3.6 ± 0.2	

The results give some evidence for a belief that the method may be used to produce better inbred lines. Richey and Sprague also studied the yields of F_1 crosses between the nonrecurrent parent and the lines recovered after successive generations of back-pollination. Except as noted, each yield given is an average of six crosses.

Cross	Yield
$(N \times R)F_2$	9.4 ± 0.6
$N \times (N \times R_2)$*	13.5 ± 0.3
$N \times (N \times R_3)$	15.7 ± 0.4
$N \times (N \times R_4)$	17.5 ± 0.4
$N \times (N \times R_5)$ (only 3 crosses)	18.3 ± 0.4
$N \times (N \times R_6)$ (only 1 cross)	17.4 ± 0.2
$(N \times R)F_1$	17.8 ± 0.5

* $(N \times R)R$ is written $N \times R_2$.

Just as the yields under continuous back-pollinating methods should approach the yield of the recurrent parent, so the yields of crosses, between unselected back-pollinated lines in different backcross generations and the nonrecurrent parent, should approach the yields of F_1 crosses as a limit according to the series of ½, ¾, etc.

It has been a common experience that backcrosses between inbred lines of corn approach the recurrent parent in appearance rather rapidly, and consequently it seems probable that two or three generations of backcrossing are all that can be used and still stand much chance of greatly changing the inbred line by the convergent-improvement plan.

Rather extensive studies of convergent improvement have been carried on by Hayes and Johnson (unpublished) at the Minnesota Station. Murphy (1941) has completed one of these studies with four inbred lines of Rustler White Dent known as C15, C16, C19, and C20. These lines were used in two single cross combinations, (C15 × C19) and (C16 × C20). Within each of these two crosses, a rather extensive convergent-improvement program was started in 1931. In 1937, after 2 years of self-pollination and selection, the recovered lines were crossed with the nonrecurrent parent and the yields of these crosses compared in replicated trials with the F_1 yields of (C15 × C19) or (C16 × C20). The yields were placed in classes of plus and minus one to five times the standard error of a difference when compared with (C15 × C19) or (C16 × C20). The results of all crosses are summarized in Table 22.

TABLE 22.—FREQUENCY DISTRIBUTION OF YIELDS OF CROSSES FOR THE RECOVERED LINES TESTED IN SINGLE CROSSES TO THE NONRECURRENT PARENT. YIELDS OF RECOVERED C16 × STANDARD C20 AND RECOVERED C20 × STANDARD C16 ARE COMPARED WITH STANDARD (C16 × C20). YIELDS OF RECOVERED C19 × STANDARD C15 AND RECOVERED C15 × STANDARD C19 ARE COMPARED WITH STANDARD (C15 × C19)

Number of lines	Years backcrossed	Class centers of minus 5 to plus 2 times the standard error of a difference							
		−5	−4	−3	−2	−1	0	+1	+2
30	2	1	2	1	4	13	5	3	1
14	3	..	2	..	1	6	4	1	
7	4	..	..	..	..	1	5	1	

Of the 51 crosses tested, 1 was placed in the yield class of +2 times the standard error of a difference more than the original F_1, and 11 crosses were in classes of −2 to −5 of the standard error

of a difference and were therefore probably significantly lower in yield than the original F_1 cross.

Seventeen F_1 crosses between recovered lines were studied in 1940. Of these 17 crosses, where both parents were recovered lines, there were 2 crosses that were placed in the +2 class and 2 in the +4 class in comparison with the original F_1 crosses. These results give some reason to believe that the yield of F_1 crosses in themselves can be improved by the method of convergent improvement. They indicate the necessity of testing the yielding ability of recovered lines, and the results show that the first test of a recovered line may be made by crossing with the nonrecurrent parent. All lines that do not yield as well in crosses to the nonrecurrent parent as the original F_1 cross may be discarded.

The improvement of an inbred line by convergent improvement seems relatively easy for those characters in which it is seriously lacking, and the other inbred carries these characters. In two cases in which one of the inbred parents in a convergent-improvement program was oustanding in smut resistance and lodging resistance and gave a good yield for an inbred and in which the other parent was deficient in these characters and gave a low yield, it was relatively easy to improve the more undesirable parent through convergent improvement and rather difficult to obtain recovered lines that were superior or even equal to the more desirable parent in yielding ability and in other important characters.

CHAPTER XV

INHERITANCE IN MAIZE

More is known regarding the genetics of maize than of any other organism except Drosophila. Some of the reasons why maize has been used extensively by students of genetics may be of interest. The plant is adapted to a wide range of environmental conditions and shows many differential characters. It is relatively easy to control pollination, and a large number of seeds can be produced on a single ear with a single pollination. There are many endosperm and seedling characters that can be studied in the laboratory and greenhouse. Technics have been developed that have made maize an especially favorable organism for cytogenetic studies.

Studies of the effects of self- and cross-fertilization with maize, which started early in the present century, have furnished the basis for the Mendelian explanation of heterosis. Later studies, with particular attention to economic characters, including the combining ability of inbred lines, have helped to give a genetic understanding of such complex characters as vigor of growth and yielding ability and have made possible the development of efficient breeding technics. In the short review given here, all that will be attempted is a brief summary of those phases of genetic studies that seem of greatest value to the student of corn breeding.

ORIGIN AND CLASSIFICATION

A rather complete review of early theories on the origin of corn and intensive recent studies have been made by Mangelsdorf and Reeves (1939). A major criterion of the probable center of origin of crop plants is the one given by Vavilov that the region of greatest diversity of type is usually the region of origin. They conclude that the wild ancestor of *Zea mays* probably occurred somewhere in the lowlands of Paraguay, northeastern Bolivia, or southwestern Brazil. Secondary centers of domestication include

the Andean region, Central America, and Mexico, where great diversity of types has been observed. Mangelsdorf and Reeves visualize maize "as a wild pod corn originating from a remote Andropogonaceous ancestor which gave rise on the South American continent to a single species *Zea mays*, on the North American continent to a more variable genus, Tripsacum."

Maize belongs to the tribe *Tripsaceae*, Hitchcock (1935), and contains three genera that are of American origin, *Tripsacum*, *Euchlaena*, and *Zea*. *Z. mays* L. contains normally 10 pairs of chromosomes and comprises a rather diverse group of varieties of Indian corn. *Euchlaena*, called teosinte, contains two species, *E. mexicana* Schrad., the annual form with 10 pairs of chromosomes, and *E. perennis* Hitchc., a perennial form of autotetraploid type with twice the chromosome number of the annual form. Extensive cytogenetic studies reviewed by Mangelsdorf and Reeves lead to the conclusion that *Zea* and *Euchlaena* do not differ widely in their chromosome make-up. In crosses between maize and teosinte, crossing-over values, with a few exceptions, which Mangelsdorf explains by differences in chromosome structure, are very similar in the hybrid to values obtained in maize. It is believed by Mangelsdorf and Reeves that teosinte was produced by hybridization between *Zea* and *Tripsacum*. They found that the major differences are a result primarily of four segments of chromatin, all bearing genes with *Tripsacum* effects. The third American genus *Tripsacum*, with $n = 18$ chromosomes, and *Zea* are believed by them to have descended from a remote common ancestor. It has been found possible to hybridize *Zea* and *Tripsacum*, and evidence indicates that they have certain genes in common, *Tripsacum* differing in its evolutionary history from *Zea* by a tendency to polyploidy accompanied by a perennial habit of growth. The wild pod maize (tunicata) believed to be the ancestor of Indian corn, presumably had its origin in South America; *Tripsacum* originated in Central and North America. It is believed that *Tripsacum* and *Zea* by hybridization and chromosomal interchange of some sort led to the development of varieties of maize that were contaminated with small additions of chromatin from *Tripsacum*. Thus, maize varieties of North America are supposed to comprise two groups: (1) pure maize, which traces its descent to wild pod corn, and (2) *Tripsacum* contaminated maize. The evidence regarding origin of many crop

plants is not very definite. The monograph by Mangelsdorf and Reeves summarizes the present status of knowledge and reviews the literature in this field. The relationships between *Zea*, *Euchlaena*, and *Tripsacum* are emphasized.

Although Sturtevant (1899) divided *Z. mays* into several groups and considered each to be of specific rank, several of the major character differences are dependent upon a single factor pair. A description of the more important groups is given here.

The Pod Corns.—Each kernel is enclosed in a pod or husks; the ear is enclosed in husks as in the other groups. The ordinary type of pod corn is heterozygous, the homozygous type being usually highly self-sterile. Mangelsdorf and Reeves have described a true-breeding pod corn bearing no ears, resulting from a combination of the homozygous condition of *Tu*, the pod-corn factor, and of Ts_5, the dominant factor for tassel seed. The rachis of the ear of pod corn is definitely more brittle than in normal maize, and there is an indication that the rachises of the tassel are more brittle also. In the true breeding form, this brittleness would aid in seed dissemination.

The Flint Corns.—The flint corns comprise varieties with a starchy endosperm in which the soft starch is surrounded by corneous starch on the outside. The relative amounts of soft and corneous starch differ widely in different varieties. Mangelsdorf and Reeves state that the original flint corn from South America was probably a small-seeded tropical form with large cobs and irregular rows. Crossing with *Euchlaena* was believed to produce the pointed popcorns, and backcrosses with tropical forms led to the development of new types of flints with straight rows.

The Popcorns.—The endosperm contains only a small proportion of soft starch, by far the major part of the starch-bearing cells carrying corneous starch. There is generally some soft starch surrounding the germ. The small size of its seeds and cobs characterizes this group. Small, hard, pointed seeds occur from crosses of maize with *Euchlaena*, and such crosses are believed to be the original source of popcorns. Several writers have shown that teosinte may be popped much like popcorn.

The Dent Corns.—The corneous starch is located at the sides of the seed, and the soft starch extends to the summit. The soft starch dries more rapidly than the corneous, which causes the

characteristic indentation of the seed. Dent corns probably originated in Mexico, and this is considered to be the center of diversity of this type. Jones (1924) obtained dent types from hybridization of Rice popcorn and Cuzco flour corn. Wallace and Bressman (1928) state that the dent corns of the corn belt probably arose from a cross between a large flint type with a late maturing type of dent that produced ears with 22 to 36 rows of rough, very soft, shoe-peg kernels.

The Flour Corns.—There is an almost complete lack of corneous starch, the group being characterized by the large amount of soft starch in the endosperm. Small amounts of corneous starch are produced by many flour corns. The location of the small amount of corneous starch determines whether the seed has an indentation.

The Sweet Corns.—This group is characterized by a translucent, horny appearance of the kernel and a wrinkled condition when dry. East (1909) concluded that sweet-corn varieties are dent, flint, or popcorns that have lost their ability to produce starch. The few starch grains produced are small and angular.

The Waxy Corns.—This group is characterized by an endosperm of waxy nature resulting from a carbohydrate of different form than in starchy varieties. The original source was China, although waxy varieties have resulted from mutation in experimental cultures.

ENDOSPERM CHARACTERS

Endosperm characters are used to differentiate several of the major corn groups. Xenia is a result of double fertilization, the following statement being quoted from Hayes and Garber:

> Xenia may result from crossing varieties which differ in a single visible endosperm character. When a character difference is dependent upon a single dominant factor, xenia occurs when the factor is carried by the male parent, or, when dominance is incomplete, xenia results when either variety is the male. When a character difference is dependent upon more than one factor, all located in one parent, and dominance appears complete, xenia occurs only when these differential factors are located in the male; when dominance is incomplete, xenia occurs if the factors are located in either parent. When two varieties have a similar character or a different character expression but contain between them endosperm factors necessary for the production of a new character, xenia occurs when either variety is the male.

A summary of the mode of inheritance of the principal normal endosperm characters is given in Table 23.

TABLE 23.—INHERITANCE OF ENDOSPERM CHARACTERS*

Parental type	F_1	Segregation in F_2
Yellow vs. colorless.....	Yellow or intermediate	3 yellow:1 colorless
Dominant white vs. yellow.	Ivory, somewhat variable in shade	3 ivory:1 yellow
Brown aleurone vs. colorless.	Pale yellow, partially dominant	3 colored:1 colorless when a single factor pair is involved
Colored aleurone (purple or red) vs. colorless.	Purple or red	Ratios 3:1, 9:7, 27:37, etc., depending on whether 1-5 factor pairs are involved
Purple vs. red aleurone	Purple	3 purple:1 red
Colored (purple or red) vs. colorless.	Colorless, because of dominant inhibitory factor	Segregation, ratios depending on number of factor pairs involved
Starchy vs. sweet.....	Starchy	3 starchy:1 sweet
Starchy vs. waxy.	Starchy	3 starchy:1 waxy
Waxy vs. sweet.....	Starchy	9 starchy:3 waxy:4 sweet
Floury vs. corneous. .	No immediate effect	1 floury:1 corneous
Normal vs. defective (various types of shrunken and shriveled).	Normal	Segregation 3 normal:1 defective when single factor par is involved

* For references to literature, see Emerson *et al.* (1935).

Emerson *et al.* (1935) have listed the genes responsible for many of the inherited characters of maize, particularly those used in linkage studies. Their monograph has been used freely in this review. There are two pairs of factors for yellow endosperm color. When both are segregating, ratios of 9 yellow: 7 white are obtained; when either is segregating in the presence of the homozygous dominant condition of the other, ratios of 3:1 are obtained. There is some evidence that genes for yellow belong to an allelic series of various shades of yellow, although it is difficult to make clear-cut classifications.

Hauge and Trost (1930) found a close physiological association in dent corn between vitamin A and the yellow endosperm. Mangelsdorf and Fraps (1931) demonstrated a direct relation

between the vitamin content of corn and the number of genes for yellow pigment in the endosperm. The average results for 2 years were as follows:

Number of genes for yellow	Factorial composition of endosperm	Units of vitamin *A* per gram
0	*yyy*	0.05
1	*yyY*	2.25
2	*yYY*	5.00
3	*YYY*	7.50

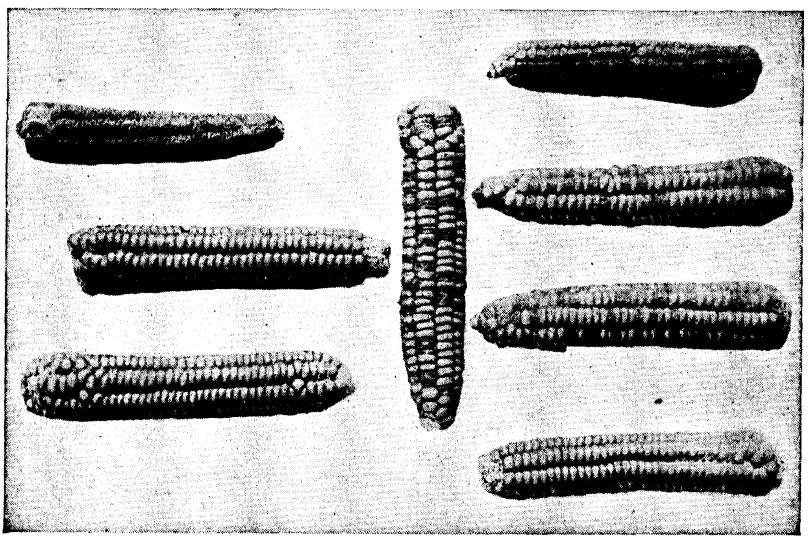


FIG. 31.—Inheritance of starchy and sweet endosperm in maize. Upper left, ear of sweet corn with wrinkled seeds; lower left, ear of flint corn with starchy seeds; left center, immediate result of pollinating an ear of the starchy parent with pollen from the sweet parent; center, an F_1 ear self-pollinated that segregated in a ratio of 3 starchy: 1 sweet; upper right, a self-pollinated ear with wrinkled seeds obtained by planting sweet seeds of the F_1. The three remaining ears at the right were produced by self-pollinating ears produced by planting starchy seeds of F_1 plants. Note that, on the average, one out of every three ears is homozygous for the starchy character. (*Photograph by East.*)

Brown aleurone, appearing as pale yellow, is dominant over colorless in the absence of purple or red aleurone. There are two factor pairs for brown aleurone, either in a dominant condition producing color in the aleurone.

There are a series of basic pigment genes designated as A_1, A_2, A_3, C, and R, all of which are necessary for production of red

aleurone color. When *Pr* also is present the color is purple. Red or purple is epistatic to brown. When a dominant inhibitor is present, called *I*, in the presence of the basic dominant aleurone factors for red or purple, the aleurone is colorless. In addition, there are several intensifying or diluting factors that modify aleurone color. There is a series of allelic factors for the *R* locus and also for the *I* locus that cause modifications of aleurone color.

In crosses of either dent or flint corn with floury, there is no immediate effect of double fertilization on the endosperm condition. Segregation on the ears of F_1 plants occurs in a 1:1 ratio. Hayes and East (1915) explained these results by the hypothesis that two genes of the floury factor are dominant over one gene for corneous and vice versa. In crosses between dent and floury, the floury segregate may show an indentation when there is a small amount of corneous starch on the sides of the kernel. Classification of floury vs. corneous is relatively easy by the use of transmitted light on a ground-glass background illuminated from below.

There are a considerable number of characters with incomplete development of the endosperm. Most of these are lethal when homozygous recessive and normal development of endosperm is dominant over defective. Mangelsdorf (1926) collected 14 defectives at random and made the necessary crosses to show that 13 of the 14 were due to different genetic factors. Sixteen different defectives have now been reported. There are also at least 15 different factor pairs that are responsible for premature germination of kernels. Certain of these give 3:1 ratios when heterozygous. There is one group of four duplicate factors that may give ratios of 3:1, 15:1, 63:1, or 255:1, depending on whether one to four pairs of factors are segregating. There are also several pairs of factors for germless seeds. Thus, it would seem that the development of normal endosperm is the result of the interaction of many factor pairs.

CHLOROPHYLL VARIATIONS

There are many recessive heritable chlorophyll abnormalities in maize. Many factors have been located in the genetic linkage map, and it is evident that there are several factors in each chromosome that in their interaction are responsible for the development of chlorophyll. These recessives are of two types,

those that appear in seedling progenies and those that appear in mature plants. In a few cases, the same factor modifies chlorophyll development in both seedlings and mature plants.

The seedling types are frequently lethal. They include eight or more white-seedling types, each the result of a single gene in the homozygous recessive condition and two cases in which duplicate genes are involved. White seedlings are devoid of chlorophyll and generally of chloroplasts; therefore the seedlings die when the food reserve in the seed is exhausted.

There are at least seven recessive genes for luteus seedlings. One of these acts only in the presence of white-seedling genes; others produce yellow seedlings in the presence of the dominant condition of a factor for white seedlings. Most luteus types are lethal; others give yellow seedlings and plants and therefore produce some chlorophyll.

Twenty virescent seedling types have been described. The seedlings are yellowish and sometimes nearly white. There is considerable variability, ranging from types that are lethal to those with normal development. The rapidity of turning to green depends upon the genes involved and on temperature and light.

There are at least 10 different genetic types of pale-green seedlings that produce a yellowish green color in the seedling. Some are lethal; others develop to maturity. In addition, about 37 other genes affect seedling chlorophyll color alone or both seedlings and mature-plant color. Thus, there are at least 86 genes that affect normal chlorophyll development in the seedling. In addition, at least 17 different genes have been described that affect chlorophyll development in the mature plant but not in the seedlings. The interaction of more than 100 genes is necessary, therefore, for normal chlorophyll development.

PLANT COLOR

There are several different plant colors that are of interest to the corn breeder. The plant and anther color resulting from the interaction of several of the genes for aleurone color with genes *B* and *Pl* for plant color (Emerson *et al.* 1935) are given in Table 24.

There is a series of alleles of a_1 that, with other factors, affect the development of plant, pericarp, and silk color that were given in

TABLE 24.—INTERACTIONS OF THE PLANT-COLOR GENES a_1, a_2, B, Pl, AND R

Gene interactions			With r^{rr}		With R^{gg}	
			Plant color	Anther color	Plant color	Anther color
A_1A_2	B	Pl	Purple	Purple	Purple	Green
		pl	Sun red	Pink	Sun red	Green
	b	Pl	Dilute purple	Purple	Green	Green
		pl	Dilute sun red	Pink	Green	Green
a_1 or a_2	B	Pl	Brown	Green	Brown	Green
		pl	Green	Green	Green	Green
a_1a_2	b	Pl	Green	Green	Green	Green
		pl	Green	Green	Green	Green

considerable detail by Emerson and others. These cannot be summarized in this short review.

A series of al eles for pericarp and cob colors is of interest. P^{rr} is the factor for red pericarp and red cob, P^{rw} for red pericarp and white cob, P^{wr} for white pericarp and red cob, and P^{ww} for white pericarp white cob. The series varies from self- or solid red through various shades of variegation, designated as P^{vv}.

GLOSSY SEEDLINGS

There are a number of different glossy seedlings that, in general, have a similar phenotypic appearance that are recessive to normal. The leaves have a glossy appearance in the early seedling stages. One of these shows the glossy character only on the third and fourth seedling leaves, whereas the usual condition is for the glossy appearance to show on the first seedling leaves. Classification is made easy by sprinkling with water from a sprinkling can, the water on glossy seedlings adhering to the leaves in large droplets. The vigor of glossies is not greatly different from normals, and the characters may be used to detect outcrosses in an inbred line.

LINKAGE STUDIES WITH MAIZE

The genes determining the characters in maize that have been studied fall into 10 linkage groups, corresponding to the 10 different chromosomes. Cytological study has demonstrated that these 10 chromosomes are morphologically distinguishable, especially at prophase in meiosis. The chromosomes are characterized by differences in total length, in the ratio of short to long arm lengths, and in the position and size of terminal or subterminal dark-staining regions. The chromosomes are numbered mainly in order of decreasing length from 1 to 10, the number 1 being the longest and the number 10 the shortest.

The independence of the 10 linkage groups has been established by both cytologic and genetic studies. In addition, the linkage groups have been identified with the particular morphological chromosomes. Thus, the longest chromosome, 1, carries linkage group 1, and the shortest, 10, carries linkage group 10. In all cases, the orientation of the linkage group within the chromosome is known, and in most cases the spindle-fiber region can be at least approximately located in the linkage map.

In the linkage map in Fig. 32, only those genes are included whose order is well established. The locus of the spindle fiber, designated S.F., must be considered to be only approximate except for group 5, genes in the part of the map above this point being located in the shorter arm of the chromosome. The terminal knob in chromosome 9 is shown. This map was drawn by C. R. Burnham from information published by Emerson, Beadle, and Fraser (1935) and from unpublished information generously supplied by several investigators.[1] The description of the characters was obtained from the same sources.

The location of the genes on the linkage map for each of the 10 chromosomes, with a description of the character produced, will be given separately for each chromosome (linkage group). In all cases, a gene symbol without subscript indicates the first or only gene with that literal symbol.

[1] Permission to use certain unpublished data in the preparation of these linkage maps of maize was given to C. R. Burnham by L. F. Randolph, A. C. Fraser, R. A. Emerson, M. T. Jenkins, E. W. Lindstrom, R. A. Brink, and H. S. Perry.

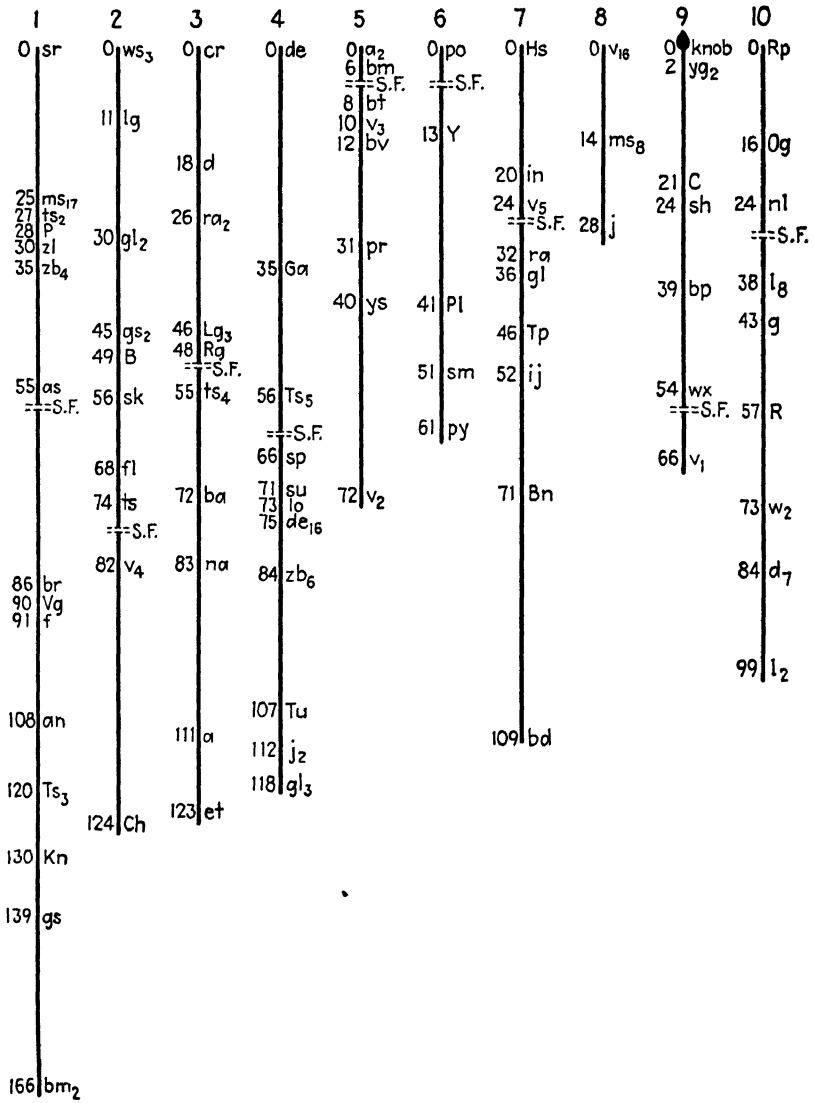


FIG. 32.—Linkage map of the ten chromosomes of *Zea mays*, showing the locus of the genes where the position has been determined with reasonable certainty. The locus of the spindle fiber, designated S.F., is only approximate in each group except group 5.

Chromosome 1.—This is the longest chromosome, physically, of the 10. The allelic series of *P*, which produces pericarp and cob colors, is located in this chromosome. The gene order and locus of 15 genes have been determined. These are listed below:

0-*sr* Striate. Leaves with fine longitudinal striations throughout the life of the plant.

25-ms_{17} Male sterile-17. Anthers usually not exserted. Some pollen occasionally shed.

27-ts_2 Tassel seed-2. Terminal inflorescence usually completely pistillate; no pollen produced. Ear develops if terminal inflorescence is removed soon after emergence. Secondary florets in ear development, giving irregular arrangement of kernels. Tassel seed-2 is similar to *ts* (chromosome 2) except that the plants are usually stronger, and terminal inflorescence is less compact.

28-*P* Pericarp and cob color. A large series of allelic genes for pericarp and cob color.

30-*zl* Zygotic lethal. Lethal that kills the very young sporophyte and endosperm.

35-zb_4 Zebra striped-4. Seedlings with irregular chlorotic crossbands on leaves in early stages. Bands may disappear as plants get older.

55-*as* Asynaptic. Partially sterile type characterized by lack of association of homologous chromosomes during the first meiotic division. Produces a few kernels when pollinated by normal plants. Usually sheds no pollen.

86-*br* Brachytic. Culm notably shortened as a result of shortening of internodes. Plants one-fourth to one-half normal height. Leaves stiff and straight.

90-*Vg* Vestigial glume. Glumes of tassel and flowering glumes of cobs greatly reduced. Anthers of tassel exposed, pollen shed only occasionally.

91-*f* Fine stripe. Seedling virescent. Plant shows fine stripes of white tissue in the leaf blades, only rarely in the auricles.

108-*an* Anther ear. Leaves broad. Plant variable in size, often almost normal in stature. Stamens develop throughout pistillate inflorescence. Ear usually ends in an unbranched spike consisting of staminate flowers only.

120-Ts_3 Tassel seed-3. Similar to *ts* and ts_2 except that the terminal inflorescence usually is mixed pistillate and staminate. Usually pollen can be obtained. Secondary florets develop in ears.

130-*Kn* Knotted leaf. Overgrowth of areas of midrib and other vascular tissue resulting in kinking or knotting of the veins.

139-*gs* Green striped. Leaves in three- or four-leaf stage, and later, show light green stripes between main vascular bundles. Plant weak.

166-bm_2 Brown midrib-2. Brown color develops in midrib and over vascular bundles of leaf blade and sheath. Similar to *bm* but less intense.

Chromosome 2.—The factor pair for flinty vs. floury endosperm (*Ff*) is located in this chromosome. The location of 10 genes in this chromosome are:

0-ws_3 White sheath-3. Partial absence of chlorophyll in culms and sheaths of seedling and older plants.

11-*lg* Liguleless leaf. Leaf usually lacks ligule and auricles and stands upright at base.

30-gl_2 Glossy seedling-2. Seedling character. Younger leaves have glossy appearance, visible in bright sunlight.

45-gs_2 Green striped-2. Mature plant with green stripes.

49-*B* Booster. Plant-color intensifier. In appropriate genotypes, gives intense sun-red, purple, or brown plant color.

56-*sk* Silkless. Pistils abort. No silks. Plants female sterile. Cobs grow normally and contain many anthers.

68-*fl* Floury endosperm. Endosperm floury (noncorneous). Female contribution to endosperm determines character; *fl fl Fl* gives floury endosperm, and *Fl Fl fl* gives normal (flinty) endosperm.

74-*ts* Tassel seed. Terminal inflorescence usually completely pistillate, no pollen produced. Ears develop if terminal inflorescence is removed soon after emergence.

82-v_4 Virescent seedling-4. Seedlings yellowish green. Plants turn green slowly and may be distinguished from normal plants later than can most virescent seedlings.

124-*Ch* Chocolate pericarp. Pericarp dark brown or chocolate in color.

Chromosome 3.—The allelic series *A*, A^b, a^p, *a* is in this chromosome. These genes are essential to the development of plant, aleurone, and pericarp colors. The 11 genes in this chromosome are:

0-*cr* Crinkly leaf. Plants somewhat shorter than normal. Leaves broad, with characteristic crinkling at base.

18-*d* Dwarf plant. Plant of very low stature, with broad thick leaves. Staminate inflorescence compacted. Stamens produced in ears.

26-ra_2 Ramose ear-2. Much less extreme than *ra* in tassel and ear.

46-Lg_3 Liguleless leaf-3. Only a portion of the ligule present.

48-*Rg* Ragged leaf. Chlorotic areas in leaves of older plants, leaves becoming much split and torn. Character shows when plants are about half-grown.

55-ts_4 Tassel seed-4. Terminal inflorescence produces staminate and pistillate flowers. Usually few kernels produced in the tassel. Secondary florets develop in ears. Pollen usually shed.

72-*ba* Barren stalk. Plant characterized by absence of pistillate inflorescence. Stem circular in cross section, lacking the characteristic groove.

83-*na* Nana. Plants dwarfed, from one-fourth to one-third normal height. Leaves characteristically stiff and twisted.

111-*a* Anthocyanin. Plant, aleurone, and pericarp color. In appropriate genotypes, gives green or brown plants, colorless aleurone and brown pericarp.

123-*et* Etched endosperm. Endosperm scarred, seedling virescent.

Chromosome 4.—The factor pair for starchy vs. sugary endosperm (*Su su*) is located in this chromosome. The factor producing tunicate plants (*Tu*) (pod corn) is also in this chromosome. The order of 11 genes has been determined. The exact location of the spindle fiber has not been determined. It is near silkless (*sk*) and is indicated on Fig. 32 by dotted lines.

0-*de* Defective endosperm. Incomplete development of the endosperm. Viability poor.

35-*Ga* Gametophyte factor. *Ga* pollen, in competition with *ga* pollen on *Ga* silks, functions in the production of 95 to 99 per cent of the kernels.

56-Ts_5 Tassel seed-5. Tassel contains both silks and anthers and is not compacted. Usually few kernels develop in tassel. Secondary florets develop in ears.

66-*sp* Small pollen. Pollen grains small but filled with starch. Transmitted usually through the ovules only.

71-*su* Sugary endosperm. Endosperm translucent and wrinkled.

73-*lo* Lethal ovule. Ovules abort. Gene transmitted almost wholly by pollen.

75-de_{16} Defective endosperm-16. Incomplete development of endosperm. Lethal.

84-zb_6 Zebra striped-6. Chlorotic crossbands in leaves of nearly mature plants.

107-*Tu* Tunicate ear. Glumes in both staminate and pistillate inflorescence long, enclosing individual kernels in ear more or less completely.

112-j_2 Japonica-2. Variegated striping. Expressed in seedlings as well as mature plants, some seedlings nearly white.

118-gl_3 Glossy seedling-3. Glossy surface on younger leaves.

Chromosome 5.—The factor pair for purple vs. red aleurone (*Pr pr*) is in this chromosome. The location of eight genes and the characters produced by them is as follows:

0-a_2 Anthocyanin-2. Dominant allele complementary to the *Aa* pair in the production of plant and aleurone colors. Has no effect upon pericarp color.

6-*bm* Brown midrib. Brown color develops in midrib and over vascular bundles of leaf blade and sheath. Character appears in three- to four-leaf stage but shows better at later stages.

S.F. Spindle fiber. Known to be between *bm* and *bt*.

8-*bt* Brittle endosperm. Endosperm translucent, usually shrunken and wrinkled.

10-v_3 Virescent seedling-3. Seedling light yellow but turns green quickly.

12-*bv* Brevis. Plants usually about one-half normal height, owing to shortening of internodes in region of pistillate inflorescence.

31-*pr* Red aleurone. In presence of other genes necessary for aleurone and scutellum color, gives red aleurone and scutellum as contrasted with purple in presence of *Pr*.

40-*ys* Yellow stripe. Leaves show yellow stripes between main vascular bundles.

72-v_2 Virescent seedling-2. Seedlings very light yellow. Plants turn green rather slowly.

Chromosome 6.—This chromosome carries the factor pair for yellow vs. white endosperm (*Yy*) and the plant-color factor pair (*Pl pl*). Five genes have been definitely located as follows:

0-*pc* Polymitotic. Plants partially sterile. Young microsphore cells undergo several mitotic-like divisions in rapid succession without division of the chromosomes. No pollen shed; few seeds produced in crosses with normal.

13-*Y* Yellow endosperm.

41-*Pl* Purple plant color. In appropriate genotypes gives dilute purple, intense purple, or brown plants.

51-*sm* Salmon silk. In presence of red pericarp (P^{rr}, etc.) silks are salmon in color. In absence of pericarp color, silks are brown.

61-*py* Pygmy. Plant short, with short, thick, striated leaves.

Chromosome 7.—The gene for brown aleurone (*Bn*) is located in this chromosome. Nine genes have been located in this chromosome. These are:

0-*Hs* Hairy sheath. Leaf sheaths hairy throughout development.

20-*in* Intensifier of aleurone color. Intensifies purple and red aleurone.

24-v_5 Virescent seedling-5. Seedlings greenish yellow, turn green very quickly.

32-*ra* Ramose ear. Ear much branched throughout, conical. Tassel much branched, conical in shape.

36-*gl* Glossy seedling. Leaves have glossy appearance.

46-*Tp* Teopod. Plant strongly tillered, with narrow leaves. Number of nodes greater than in normal plants. Many small podded ears. Staminate inflorescence with long bracts, many plants not shedding pollen.

52-*ij* Iojap striping. Variegated stripe that shows throughout life of the plant. Varies from albino to variegated.

71-*Bn* Brown aleurone. Pale yellowish aleurone color. Shows only in absence of purple and red aleurone. Often confused with light yellow endosperm.

109-*bd* Branched silkless. Ears branched at base, often without silks. Tassel has characteristic branches, the spikelets occurring in groups of more than two.

Chromosome 8.—Fewer genes have been located in this chromosome than in any other. Of the three genes known to be in this chromosome, all are in the long arm. Consequently, the spindle fiber is not shown in Fig. 32. The order of the known genes is as follows:

0-v_{16} Virescent seedling-16. Seedlings yellowish green.

14-ms_8 Male sterile-8. No anthers exserted. Microsporocytes usually disintegrate.

28-*j* Japonica. Variegated striping in leaves and sheath. Does not show in seedling stage.

Chromosome 9.—One of the basic aleurone color factors (*C*) is located in this chromosome. So is the gene for waxy endosperm (*wx*). This chromosome, in certain stocks, has a terminal knob at the end of the short arm. Six genes have been placed in order on this chromosome, five being in the short arm. These are as follows:

0-knob Terminal knob on the chromosome.

2-yg_2 Yellow green-2. Seedling and plant yellowish.

21-*C* Aleurone color. In appropriate genotypes, gives purple or red aleurone.

24-*sh* Shrunken endosperm. Endosperm shrinks during drying stage at maturity, giving a smooth indentation at the crown or a collapse at the sides of the kernel.

39-*bp* Brown pericarp. In presence of *P*, gives brown pericarp.

54-*wx* Waxy endosperm. Waxy starch in endosperm; embryo sac and pollen grains stain reddish brown with iodine solution, as contrasted with normal starch, which stains blue.

66-*v* Virescent seedling-1. Seedlings yellowish, become green relatively early in development.

Chromosome 10.—This is the shortest chromosome, in terms of physical length, of the 10. It has a genetic map length of 99 units. One of the basic aleurone and plant-color factor pairs (*Rr*) is located in this chromosome. The locations of 8 genes have been mapped.

0-*Rp* Resistance to leaf rust. Resistance to physiologic race 3 of *Puccinia sorghi*.

16-*Og* Old gold. Dominant chlorophyll striping. Light-green or yellow striping begins after 5- 6-leaf stage.

24-*nl* Narrow leaf. Plants weaker than normal, with narrow leaf blades. Leaves tend to be longitudinally striated, like lineate (*li*).

38-l_8 Luteus-8.

43-*g* Golden. Full-grown plants of a yellowish green color.

57-*R* Colored aleurone and plant. In appropriate genotypes, gives purple or red aleurone. Exists in a series of alleles affecting aleurone, plant, and anther color.

73-w_2 White seedling-2. Seedling albino, devoid of chlorophyll.

84-d_7 Dwarf-7. Plant of low stature.

99-l_2 Luteus-2. Yellow seedling.

In addition to the 86 genes whose location on the chromosome map has been determined with reasonable certainty, there are about 108 other genes that have been placed in particular chromosomes, although the location on the map, in relation to the genes whose locus is known, has not been determined. Some 102 chromosome translocations have been found in which the chromosomes involved have been determined.

INHERITANCE OF QUANTITATIVE CHARACTERS

Studies on the inheritance of quantitative characters in maize were started by East, in 1906, in Connecticut, and a little later by Emerson, in Nebraska. These and other experiments created a wide interest in the multiple-factor explanation of the inheritance of size characters. It is rather generally accepted that many normal characters are the result of the interaction of many genetic factors. A method commonly used with size characters is to cross parents that differ rather widely in a character, such as length of ear in maize, and study the F_1, F_2, and F_3 generations in comparison with the parents.

For quantitative characters, dominance is often incomplete or lacking. When dominance is complete, the expected ratios may be obtained by the expansion of the binomial $(3 + 1)^n$, where n is the number of allelic pairs of factors. When the heterozygous condition of a factor pair gives half the effect of the dominant homozygous condition and there is a cumulative effect of one factor on another and all factor pairs are of equal value in their effect on the character, the expected ratios in F_2 may be obtained by the expansion of the binomial $(1 + 1)^{2n}$. Where n

is 3, for example, or three factor pairs are involved, the expected ratio will be 1:6:15:20:15:6:1.

Such a ratio approaches the normal curve, and when sufficient F_2 individuals are studied the parental combination of characters should be recovered. If each of the parents contains different factors that have an effect on the character, illustrated by the cross of *aaBB* × *AAbb*, types will be obtained in F_2 and later generations that exceed the limit of the parents. In actual practice, there is no reason to expect that all factors have like value in their effect on the character. This will affect the form of the curve but not its regularity in the absence of dominance. With partial to complete dominance, the curve will be of the skew types but cannot easily be distinguished from normal when a large number of factor pairs are segregating.

An illustration of the usual type of data that are obtained, where dominance is incomplete, may be observed from a cross between Tom Thumb pop with Black Mexican sweet, as given by Emerson and East (1913).

TABLE 25.—FREQUENCY DISTRIBUTION FOR LENGTH OF EARS IN THE PARENTS F_1, F_2, AND F_3 GENERATIONS OF A CROSS BETWEEN TOM THUMB POP AND BLACK MEXICAN SWEET CORN

Parent or cross	Generation	Parent class	Ear classes, cm																	Mean
			5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Tom Thumb	*P*	..	4	21	24	8	..	..	..	..	...	..	..	.			.	..	.	6.6 ± .07
Black Mexican	*P*	..	.	..	.	..		..		..	3	11	12	15	26	15	10	7	2	16.8 ± .12
	F_1	..	.	..	..	..	1	12	12	14	17	9	4	..	..	.	.	.		12.1 ± .12
	F_2	..	.	.	4	5	22	56	80	145	129	91	63	27	17	6	1			12.7 ± .06
	F_3	11	.	..		..	1	5	8	9	25	20	2	..	.	..		..		12.7 ± .11
	F_3	18	.	..		..	..	..		9	7	13	18	25	20	6	4	..	.	15.8 ± .13
	F_3	10	.	1	.	8	22	25	15	22	7	2	.	..	.	.	.	.		10.5 ± .10
	F_3	9	.	5	13	20	28	28	30	10	7	6	3	2	1	..		..		10.0 ± .12
	F_3	10	.	3	10	33	47	40	18	3	...	.	..	.	..		.	..	.	9.2 ± .07

From these results, it will be noted that the F_1 was intermediate in ear length but no more variable than the parents and that the F_2 was more variable than the parents. The few F_3 lines illustrated give wide differences; the shortest eared line had a mean of 9.2 ± .07 and the longest, 15.8 ± .13. If sufficient lines had been tested, it is reasonable to expect that the parental forms

would have been recovered, the frequency of such recovery depending upon the number of factor pairs involved and the nature of their interaction.

Similar data were given by Emerson and East for diameter of ears, weight of seeds, breadth of seeds, height of plants, number of nodes per stalk, internode length, number of stalks per plant, total length of stalks per plant, and duration of growth. For these quantitative characters, it should be emphasized that when several pairs of factors are involved it may be difficult to recover the parental types in F_2. The usual method used by the breeder is to grow as large a population in F_2 as can be studied and select for the character desired. Recovery of the parental types may be obtained, as a rule, by continuing selection in F_3 and in the later segregating generations.

LINKAGE OF FACTORS FOR ROW NUMBER WITH GENES AT KNOWN LOCI

Further proof of the multiple-factor explanation of the inheritance of quantitative characters has been obtained by studies of

TABLE 26.—COB COLOR AND ROW NUMBER IN THE F_2 OF THE CROSS OF IODENT × GOLDEN BANTAM AND IN THE BACKCROSS

Rows per ear	F_2 generation		F_1 × Golden Bantam	
	R*	r	R	r
8			24	62
10	3	4	94	109
12	40	17	116	101
14	46	6	19	10
16	17	3	3	0
18	2	0		
Total.....	108	30	256	282
P	0.03		0.0001	

* In this table, R stands for red cob and r for white.

linkage between quantitative characters with genes that are known to be located in particular loci of the chromosome map. Studies have been made by Lindstrom (1931) of the linkage relations of row number with the gene P for pericarp and cob color, the R factor for aleurone color, Su for starchy endosperm,

and *Y* for yellow endosperm color. An illustration of the type of results obtained will be given for a cross of Iodent with a modal value of 16 rows per ear and red cob × Golden Bantam, an 8-rowed, white-cobbed variety. The extent of association was determined by the calculation of X^2 and *P* for independence.

In both F_2 and the backcross, it is evident that the white-cobbed (*r*) ears average lower in row number than the red-cobbed ears. The data given illustrate one of a rather extensive series of crosses where there appeared to be an association between row number and cob color. This linkage relation seems best explained by the hypothesis that one of the factor pairs for row number is located on chromosome 1 and shows genetic linkage with one of the allelic pairs of factors for pericarp and cob color.

In studies of linkage relations between row number and the factor pair *Su su* for starchy-sugary endosperm, crosses were made in both phases, *i.e.*, high row, sugary × low row, starchy and low row, sugary × high row, starchy. In certain crosses of both types, there was definite evidence of genetic linkage; other crosses did not show association.

With the *Yy* factor pair, there was some evidence of a loose linkage. The greater part of the data also showed evidence of linkage between the factor pair for aleurone color *Rr* and row number.

INHERITANCE OF SMUT REACTION

Studies of linkages for smut reaction have been made by various workers. Immer (1927) and Hoover (1932) made crosses between resistant inbreds and genetic testers that were susceptible. Most of the cases of association were between characters such as tassel seed, brachytic and liguleless, and susceptibility. These are of such a nature that the association may be explained on the basis that the morphological character tends to make the plant more susceptible. Immer observed an association between the pericarp factor pair *Pp* and smut reaction; Hoover obtained evidence in certain crosses, but not in others, of linkages between smut reaction and *su*, v_2, and *sh wx* located in chromosomes 4, 5, and 9, respectively.

More recently, studies of linkage of smut reaction have been made by the use of chromosome interchanges. In these experiments, smut-resistant inbred lines were crossed with particular

interchanges that were smut-susceptible. In the studies of Burnham and Cartledge (1939), the F_1 was rather highly resistant, and this was outcrossed to a normal susceptible inbred. Interchange plants can be differentiated from normal, since they produce approximately 50 per cent aborted pollen grains. In the experiments of Saboe and Hayes (1941), the F_1 cross between a resistant inbred and a susceptible interchange was intermediate in susceptibility. The F_1 was backcrossed to the resistant inbred parent.

In both experiments, the plants in the segregating families were first classed as normal or interchange on the basis of pollen sterility, and data were taken on smut reaction. A portion of Burnham and Cartledge's results will be summarized to show how linkages were determined.

TABLE 27.—REACTION TO SMUT IN THE PROGENY OF BACKCROSSES OF THE F_1 (RESISTANT INBRED × SUSCEPTIBLE INTERCHANGE) × SUSCEPTIBLE NORMAL

F_1 cross	Normal		Semisterile		P
	Smutted	Not smutted	Smutted	Not smutted	
1–2*a* × resistant	57	215	55	198	0.80
1–2*c* × resistant..	53	261	91	257	<0.01
3–8*a* × resistant	210	602	250	488	<0.01
1–9*c* × resistant.	10	38	17	34	0.20

Highly significant deviations from random expectation were obtained with interchanges involving 1–2*c* and 3–8*a*, as shown in the table, and also with 1–6*a*, 1–9*b*, 2–6*a*, and 6–8*a*. In the case of the interchange 1–2*c*, where definite linkage is noted, and the point of interchange is close to 1–9*c* in chromosome 1, where there was no evidence of linkage, it seems probable that the linkage relation is with chromosome 2. Break 1–2*c* occurred near the location v_4 in the longer arm of chromosome 2.

In the studies by Saboe and Hayes, significant associations were observed with interchanges 3–7*b*, 5–7*d*, 6–9*a*, and 8–10*a* in the crosses of interchanges with the resistant inbred line of Minn. 13 and with interchanges 1–4*a*, 3–5*c*, and 5–8*a* in crosses with the resistant inbred from Rustler.

From these results, it is evident that there are many loci for reaction to smut. Various investigators, including Jones (1920), Hayes *et al.* (1924), and Garber and Quisenberry (1925), have found it relatively easy to isolate resistant inbred lines by selection in self-fertilized lines. It is possible that the number of factor pairs for smut resistance is not necessarily very great from any one source of origin.

INHERITANCE OF COMBINING ABILITY

There is a great deal of information that leads to the conclusion that some inbred lines combine well in top-crosses or with most unrelated lines, whereas other inbreds rather generally have lower combining ability. This entire problem has received some consideration in the chapter on Breeding Methods, but some of the more salient facts in relation to inheritance of combining ability will be reviewed briefly. Experiments at Minnesota and Iowa, already reviewed, show that there is a direct correlation between the vigor of inbred lines and their yielding ability in top-crosses. It is equally evident that the relationship is not easily measured by the eye, since it is impossible to determine by inspection whether a particular inbred will give high or low yields, on the average, in crosses.

There is general acceptance that the yielding ability of inbreds, as determined by their crosses, is dependent upon the number and nature of dominant growth factors of each inbred in relation to the dominant factors carried by the other parent. This has led to the test of inbred lines for their combining ability and the selection of genetically diverse lines to use in any particular hybrid. Davis (1929) first suggested the use of top-crosses to test combining ability, although the general wide use of the method should be credited to the work of Jenkins and Brunson (1932). Wu (1939), Hayes and Johnson (1939), and Johnson and Hayes (1940) have summarized extensive studies that show that yielding ability in single crosses is greater, on the average, in lines that are unrelated on the basis of origin than in lines of a somewhat similar origin. Using inbreds of diverse origin that had been classified on the basis of combining ability in top-crosses into two groups for yielding ability, low and high, Johnson and Hayes (1940) found that, on the average, crosses between low $\times$ low yielded less than low $\times$ high or high $\times$ high, although F_1

crosses between low × high yielded as well, on the average, as F_1 crosses between high × high.

Eckhardt and Bryan (1940) selected inbreds from two different varieties and called those from one variety A and B and from the other, X and Y. From any double cross, where two inbreds were selected from one variety and two from the other, the yield of $(A \times B) \times (X \times Y)$ was significantly greater than $(A \times X) \times (B \times Y)$ or $(A \times Y) \times (B \times X)$.

Several studies on the inheritance of combining ability have been made. Jenkins (1935) concluded that inbred lines showed their individuality as parents in top-crosses in the early segregating generations and remained relatively stable in later inbred generations. This has led to a consideration of the value of testing combining ability in the early generations of selfing and continuing selection in self-pollinated lines from lines of high combining ability. Jenkins explained these results on the basis that combining ability was controlled by a large number of dominant genes and that the effect of different genes was of approximately the same value. He thought equal numbers of favorable dominant genes would be preserved by chance through the successive generations of selfing.

In a recent study, Jenkins (1940) selected seven inbred lines of the variety Krug that had been tested in top-crosses for each of 4 successive years, the tests having been begun after the lines had been selfed for 3 years. The results of these trials are as follows:

Inbred	Acre yield in top-crosses				
	1930	1931	1932	1933	Mean
K679	39.7	75.8	71.3	80.9	66.9
K682	37.3	74.6	81.8	92.3	71.5
K683	51.9	81.1	77.6	84.3	73.7
K685	22.4	70.4	74.7	79 2	61.7
K686	37.9	79.8	79.5	86.3	70.9
K687	31.1	79.5	66.1	73.6	62.6
K689	36.2	76.4	71.7	82.0	66.6
Krug variety	37.5	76.5	75.1	79.6	67.3

Remnant seed of the first-year selfs S_1 was used for each line, and within each line pollen of each of 16 plants was applied to the

silks of 25 plants of the Krug variety. Seed for each top-cross was obtained by mixing the seed of 25 ears, and the 112 top-crosses so made were tested in replicated yield trials. The analysis of variance is as follows:

TABLE 28.—ANALYSIS OF VARIANCE OF YIELDS OF TOP-CROSSES OF INDIVIDUAL PLANTS IN ONE-GENERATION KRUG SELFS

Source of variation	Degrees of freedom	Mean squares	F
Lines	6	680 32	34.07*
Sibling plants within lines	105	77 21	3.87*
Replications within lines	63	403 45	20.20*
Error	945	19 97	
Total	1119		

* Highly significant.

On the basis that heterozygosity, and therefore variance, will be reduced among siblings within lines in the various succeeding generations of selfing according to the series ½, ¼, ⅛, etc., Jenkins determined the probable significance of segregation for combining ability. The variance for sibling plants within lines in the first selfed generation was 77.21. This is composed of error mean square 19.97 and variance due to genetic differences or 57.24. The calculated mean squares for sibling plants in the various generations of selfing from S_1 to S_8 were S_1 77.21, S_2 48.59, S_3 34.28, S_4 27.13, S_5 23.55, S_6 21.76, S_7 20.86, and S_8 20.42, respectively. Although all generations except S_8 showed highly significant calculated variations, it is apparent that the chances for segregation were much the greatest in the early generations of selfing and on the average became progressively less as selection progressed.

Sprague and Bryan (1941) studied segregation for yield, lodging, and damaged kernels in top-crosses of 73 F_3 lines selected from a single cross between inbreds. Twelve F_3 lines were selected that represented low, medium, and high yielders in top-crosses and that differed in damaged kernels and lodging resistance. Five F_4 plants were chosen from each F_3 line and top-crossed to the synthetic hybrid 8037 and tested in yield trials for 1938 and 1939. Highly significant variances between F_4 lines within F_3 families

were obtained for yield and lodging, and a significant variance on the basis of odds of 20:1 was obtained for damaged kernels.

Hayes and Johnson (1939) studied 110 inbreds obtained from selection through F_6 in the progeny of single crosses between inbred lines. These crosses comprised three general types of crosses between inbreds that were classified as low and high combiners in top-crosses. From crosses between low × low, most of the inbreds selected proved low in combining ability. Crosses of low × high combiners and subsequent selection through F_6 gave both low and high combining lines, whereas from crosses of high × high and subsequent selection in selfed lines through F_6 only high combining lines were isolated.

INHERITANCE OF OTHER IMPORTANT CHARACTERS

A complete review of important studies of inheritance in maize cannot be made in the space available. Inheritance of protein content has been studied by Hayes and Garber (1919), East and Jones (1921), and Hayes (1922). Crosses between low and high protein lines have low protein content in F_1. It seems probable that many genes are responsible for the inheritance of protein. East and Jones concluded that the apparent dominance of low protein over high protein was a result of heterosis, which is a further indication of the multiple-factor theory of protein inheritance.

Jenkins (1932) has shown that inbred lines and crosses in corn have differential resistance to heat and drought. Haber (1938) obtained similar results with inbred strains of sweet corn. Heyne and Brunson (1940) have found also that selfed lines of corn can be isolated that differ in reaction to heat and drought. Heat tolerance was definitely inherited and usually intermediate to dominant in F_1. A case of linkage of reaction to heat with the *Pr pr* factor pair was noted. The *su* gene was directly responsible for susceptibility to heat while certain of the glossy seedling genes *gl* and gl_2 apparently protected the seedlings from heat.

Holbert and Burlison (1928) noted marked differences in reaction to cold between inbred lines and within commercial varieties.

Most maize plants are proterandrous, the pollen being shed 3 to 5 days before the silks appear. A variety of popcorn from Spain was found to have proterogynous habit, the pollen being shed 2 or 3 days after the silks emerge, which is the normal condi-

tion in *Tripsacum* and *Euchlaena*. The inheritance of this character has been studied by Kempton (1924). The proterogynous strain used in crosses averaged 2.96 ± 0.18 days from silking to pollen shedding; the proterandrous strain shed pollen 2.3 ± 0.11 days before the silks appeared. No proterandrous plants were found in the proterogynous strain. The proterogynous strain produced an occasional plant that showed a tendency to be proterandrous. The proterogynous strain also produced several plants that failed to extrude anthers and never shed pollen. In crosses between the two strains, the F_1 was proterandrous. Segregation occurred in F_2, the number of proterogynous plants obtained being too few for a simple Mendelian ratio. Male sterile plants appeared also, and the conclusion was reached that proterogyny was a result of a variable expression of the male sterile condition brought about by modifying factors.

Pericarp tenderness has been found by Johnson and Hayes (1938) to be an inherited character. Different inbred lines give wide deviations for the mean expression of tenderness. The number of factor pairs involved was not determined, but the results proved that it was relatively easy to modify the tenderness of an inbred line of sweet corn by a process of crossing, backcrossing, and selection.

Harvey (1939) has reviewed previous studies of differential responses of corn to various levels of fertility. The various studies show clearly that inbred lines and their F_1 hybrids frequently show differential response to various nutrients, including phosphorus and nitrogen, and in water economy. Harvey dealt with the absorption and utilization of nitrogen ionic forms by corn inbreds and hybrids. The inbred strains and their F_1 hybrids were grown in aqueous mineral solutions. Differential response to ammonium and nitrate nitrogen was statistically significant. Some strains made relatively more growth than other strains on ammonium nitrogen compared with their growth on nitrate nitrogen. The response of F_1 crosses indicated that there was a partial dominance of the genetic complex for efficient utilization of ammonium nitrogen.

There are wide differences among varieties, inbred lines, and hybrids in reaction to important diseases and insect pests. Mains (1931) studied reaction to leaf rust, *Puccinia sorghi*, using physi-

ologic races 1 and 3. Resistance to both races was due to the same genetic factor. In crosses of resistant × susceptible, resistance was dominant, and segregation in F_2 was on the basis of 3 resistant: 1 susceptible. Ivanoff and Riker (1936) and Wellhausen (1937) have shown that resistance to bacterial wilt was an inherited character. In general, in crosses of resistant with susceptible inbreds, resistance behaves as a dominant. Wellhausen concluded that there were at least three pairs of factors, independently inherited, that condition resistance. The presence of all three dominant factors in either a heterozygous or a homozygous condition resulted in a high degree of resistance. Differences in reaction to ear, stalk, and root rots have been observed by many investigators. The mode of inheritance has not been worked out in detail.

Resistance to insect pests has been studied by several workers. The leaf aphid, *Aphis maidis* Fitch, attacks the strains of susceptible plants and prevents pollen shedding. Snelling *et al.* (1940) reviewed the literature on resistance to aphis attack and presented data on inbreds and crosses between them to show that resistance to aphis injury is a heritable character. There have been numerous studies of resistance to the European corn borer. Marston (1930) studied crosses of Maize Amargo, a resistant variety, with Michigan varieties and concluded that reaction to the borer segregated in a ratio of 3 susceptible: 1 resistant. Meyers *et al.* (1937) found resistance to be an inherited character but conclude "nothing suggestive of immunity nor of a genetically simple resistance was found." Inherited resistance to the corn-ear worm was reported many years ago by Collins and Kempton (1917), and considerable progress has been made in the development of resistant varieties. Blanchard *et al.* (1941) found that some inbred lines were resistant to the corn-ear worm, whereas others were susceptible. Some resistant inbreds transmit a high degree of resistance to their F_1 crosses with either resistant or susceptible inbreds. Other F_1 crosses of resistant × susceptible did not show a dominance of resistance. F_1 crosses of susceptible inbreds were generally susceptible, although one case of a resistant F_1 was obtained from a cross of susceptible inbreds. Marked differences in reaction to the chinch bug have been noted by Snelling and Dahms (1937).

CHAPTER XVI

CONTROLLED POLLINATION METHODS OF BREEDING CROSS-POLLINATED PLANTS

Darwin (1876) made the first carefully controlled extensive experiments of the effects of self-fertilization. He noted the great uniformity of inbred lines and in general a marked reduction in vigor in self-pollinated lines, although he recorded exceptions. In several cases he found little harmful effect of continued self-fertilization after the first generation. He observed that continued brother-sister mating had the same effect as continued self-fertilization. He believed, however, that this similarity was the result of growing the inbred cultures under the same environmental conditions, for he found that crosses between his inbred stocks and those from another locality were very vigorous.

In spite of these results, Darwin agreed with Knight that self-fertilization was not a natural process. They were the chief exponents of the so-called Knight-Darwin law that "nature abhors perpetual self-fertilization." The vigor of F_1 crosses was explained by Darwin on the basis of germinal differences contributed by the parents.

EFFECTS OF SELF-FERTILIZATION

East and Jones (1919) summarized many of the experiments on the effects of inbreeding and on hybrid vigor and gave what seems to be a sound biological explanation of the results. This monograph furnishes a wealth of information for the student of plant breeding.

In its application to plant and animal improvement, inbreeding gives an opportunity for controlled selection and in this way aids in the rapid isolation of strains homozygous for the desired characters. In many cases, the vigor of growth of a plant or animal is dependent upon the interaction of a large number of growth factors. Most of these factors are dominant or partially domi-

nant in hybrids, and, because of their number, linkage is involved. Inbreeding tends to reduce the number of heterozygous pairs of growth factors present in the inbred line of the organism.

In self-pollinated crops, natural and artificial selection has led to the development of vigorous inbred lines. It would seem that artificial inbreeding and selection with cross-pollinated crops might be expected to accomplish similar results. Continued studies of the effects of inbreeding and selection with cross-pollinated crops plants show the value of the methods, although there are many instances where the reduction in vigor is so great that inbreeding cannot be continued for many generations with the hope of obtaining inbred lines that are vigorous.

Studies of self-fertilization with corn and other organisms, since the rediscovery of Mendel's laws, have furnished the basis for the Mendelian explanation of hybrid vigor and the partial standardization of breeding technic with cross-pollinated plants. The extent to which controlled inbreeding can be used, the desirability of breeding by adding the factor for self-fertility to many lines when it is available in the organism and where extensive self-sterility is involved, as well as many other similar problems, can be solved only by extensive study with each particular crop plant. A brief review of the effects of self-fertilization with several different crop plants will serve to show the wide diversity of results when selfing is practiced and indicate the difficulty of a close standardization of breeding methods. It seems probable that such standardization will depend, in a large measure, on the effects of self- and cross-fertilization with the crop plant in question.

As has been emphasized in Chaps. III and XIV, it is probable that more information is available on the effects of self-fertilization in corn than for any other cross-pollinated plant. In general, all inbred lines of corn obtained so far are less vigorous than normal corn, although some inbred lines are relatively vigorous and normal in habit of growth. Inbred lines differ widely in resistance to diseases, such as bacterial wilt in sweet corn and reaction to smut, as well as ability to withstand environmental conditions generally considered unfavorable. The most noticeable effect of inbreeding in corn, in addition to reduction of vigor and the isolation of lines that are relatively homozygous, is the appearance of many recessive abnormalities.

Studies of inbreeding with alfalfa have been made by Kirk (1927, 1932, 1933). In general, the loss in vigor is rather great when alfalfa is self-fertilized for several generations. Kirk says, "The results of selfed line breeding have not been impressive as a practical method of improvement." H. M. Tysdal, of the U.S. Department of Agriculture, cooperating with the Nebraska Agricultural Experiment Station, has continued self-fertilization with alfalfa for a greater number of generations than has been reported by any other investigator. The results are given here in considerable detail and may be compared with those for corn that have been discussed in Chap. III. From three trials under Nebraska conditions using, in two of the three studies, lines of alfalfa with known genetic characters, Tysdal concluded, "As an average of the three tests under open-pollinated conditions, 89.1 percent natural crossing was found." This is somewhat lower than in corn but higher than has usually been reported for alfalfa.

The following statement from Tysdal (1941) describes the results of a study of the effects of selfing on forage and seed yields:

> From the amount of natural crossing found in alfalfa, it would be expected that self-fertilization would lead to a reduction of vegetative growth and seed yield. The selfing program has been included for a number of years as a part of the alfalfa improvement and breeding program at Nebraska. While most of the lines have been selfed for only one or two generations, a few have been carried into the seventh and eighth generations of inbreeding. A number of these lines were planted in space-planted nurseries with the rows spaced 27 inches apart and the plants separated by 18 inches in the row. The self-fertilized lines were planted in comparison with hybrids between these lines, their open-pollinated progeny, and the standard varieties, Grimm, Ladak and Hardistan, the latter belonging to the Turkestan group, which represented the varieties from which practically all of the inbred lines originated.

Yields of seed and of forage were obtained in terms of the average yield of the three varieties. The forage yields were obtained by taking green-weight yields on a 2-year basis and the seed yields for a single season. Results given in Table 29 are an average for the number of lines tested, all lines containing at least 10 plants. Usually 30 to 60 plants formed the basis for taking yields.

TABLE 29.—YIELDS OF SELF-FERTILIZED LINES OF ALFALFA IN PERCENTAGE OF THE PARENTAL OPEN-POLLINATED VARIETIES GRIMM, HARDISTAN, AND LADAK*

Number of selfed generations	Number of lines tested	Actual yield in per cent of original parents		Theoretical yield	
		Forage	Seed	Forage	Seed
1	54	68	62	68	62
2	17	48	39	52	43
3	9	59	38	44	33 5
4	13	51	36	40	28.75
5	1	41	29	38	26 37
6	..	..	.	37	25.18
7	1	26	15	36 5	24 58
8	4	28	8	36 25	24 28

*Courtesy of H. M. Tysdal.

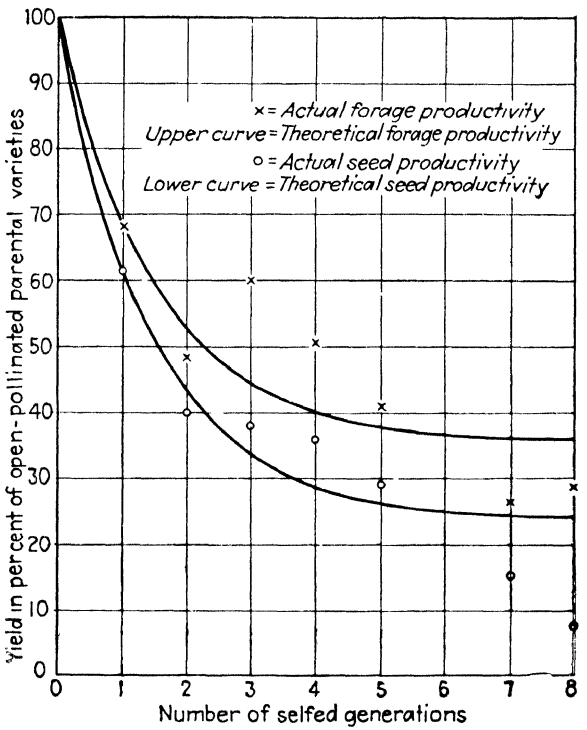


FIG. 33.—Point diagram showing average forage and seed yields of self-fertilized lines of alfalfa after one to eight generations of inbreeding, and curves showing theoretical decrease in yield of self-fertilized lines. (*Courtesy of H. M. Tysdal.*)

The theoretical yields given in the table were calculated by comparing the average yields of the open-pollinated varieties Grimm, Hardistan, and Ladak as 100 per cent with the yields of 54 first-year selfed lines at 68 per cent, giving a reduction of 32 per cent and the expectation that yields would be reduced one-half in each succeeding generation. For example, subtracting one-half of 32, or 16, from 68 gives an expected yield for second-generation selfed lines of 52 per cent. These results are given in the diagram in Fig. 33. Although selection seemed to lead to a slowing up in the reduction in yields in the first five selfed generations, further years of selfing caused a greater reduction in yields of both seed and forage than the theoretical expectation.

In discussing these results, Tysdal says, in part:

It would be difficult if not impossible to give an exact curve of reduction in yield caused by inbreeding in alfalfa because it would be necessary to consider the origin of the material as well as to very carefully refrain from any type of selection whatever. Obviously, selection is practiced among the inbred lines in a breeding and improvement program and, therefore, the results presented above are subject to whatever bias may result from such selection. In some cases, lines were carried for the purpose of determining the principle rather than for selective purposes, but on the other hand, some were eliminated in the selection program while still others reduced so rapidly in seed yield that they could not be carried at all. To indicate the wide range in forage yield of selfed lines, for example, it is only necessary to point out that the S_1 lines varied from 26 per cent to 105 per cent. Seed yield is even more variable in selfed lines than forage yield. Some lines in advanced generations showed increased productivity over the original parent, while others decreased very rapidly. This divergence might be attributed, at least to some extent, to the variability in seed setting in alfalfa in general, and also perhaps, to the peculiarities of the conditions under which the test is made. Those lines which might be selected for high self-fertility, as autogamous lines, for example, might produce unusually well under conditions of limited cross pollinating insect activity. The origin of the selfed lines no doubt also plays an important part. Selfed lines from Turkestan origin apparently do not reduce as rapidly as those from Grimm or Ladak origin. In general the Turkestan group appears to be more homozygous than alfalfas of hybrid origin such as Ladak and Grimm, and it may be that the diversity of origin of the latter would produce a greater range and possibly a different type of curve in yields of inbred progenies. Further, when a given plant is

chosen for selfing from a mass population, there is no way of knowing whether it, itself, was the result of cross- or self-fertilization.

The results obtained with alfalfa "are remarkably similar to those obtained in corn and this together with the fact that hybrid vigor has been demonstrated in alfalfa similar to that in corn (unpublished data) leads to the conclusion that the principles of breeding in this crop are essentially the same as those which have been established for corn."

With rye, Heribert-Nilsson (1916, 1919, 1921) found 1 or 2 plants out of 100 that were highly self-fertile, although self-sterility was the usual condition. Some inbred strains approached in yielding ability the normal variety from which they were obtained. The number of recessive abnormalities is somewhat less in rye than in corn. Brewbaker (1926) believed self-fertilization and selection a desirable method of breeding rye, although from studies that have been continued at Minnesota no inbred lines have been obtained as vigorous as normal. Peterson (1934) studied crosses between self-fertile and self-sterile inbred lines and found that the factor for fertility bred out the sterility alleles in later generations of selfing from a cross between fertile and sterile lines. Whether self-fertility is desirable or undesirable in rye is an unanswered question.

With sunflowers, Hamilton (1926) found a reduction in vigor, after selfing in most lines. He says, "Unlike the inbred strains of corn, however, a number of the sunflower strains, while becoming extremely uniform, did not lose any of their former vigor. In fact some of the tallest, leafiest and highest yielding rows under test during the past five years were strains that had been inbred for five consecutive generations."

In timothy breeding, as developed at Cornell, selfing was practiced for a year and the better types then used for breeding stocks. Clarke (1927), at Minnesota, concluded that vigorous lines of timothy could be obtained without great difficulty and believed that self-fertilization and selection in self-fertilized lines was a practical means of breeding. Valle (1931), in a breeding study in Finland, states that the percentage of self-fertile and self-vital lines was too low to make self-fertilization and selection a valuable method of breeding timothy.

Jenkin (1931*b*), at Aberystwyth, placed timothy in two main groups, the hay type, with 42 chromosomes, and the pasture type,

with 14. Self-fertility was variable, the pasture type being much less self-fertile than the hay type. Self-sterile plants are frequent in commercial varieties of American timothy, although self-fertility is common also. Two strains of timothy have been

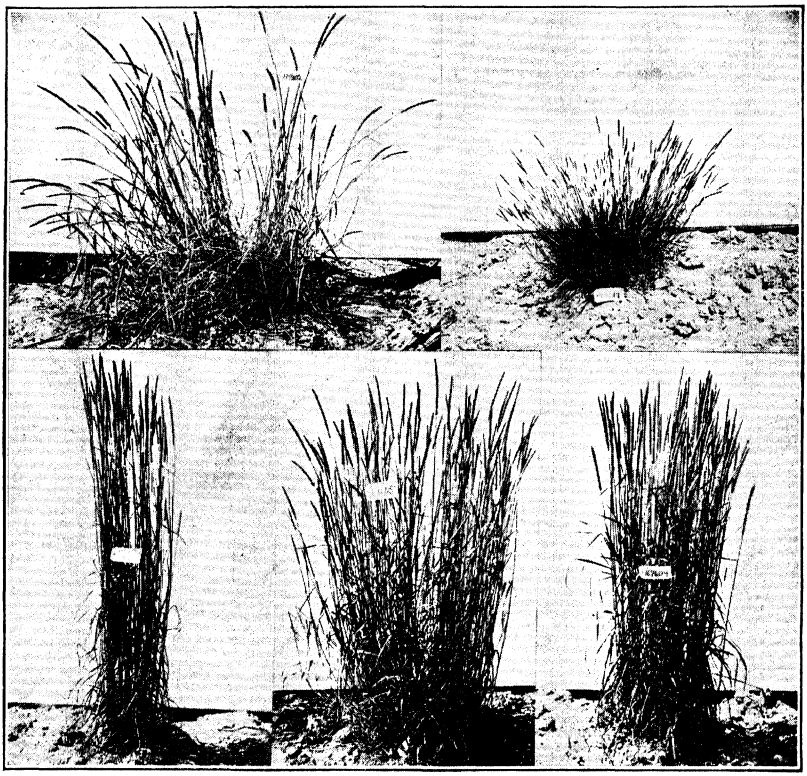


FIG. 34.—Individual timothy plants grown under like conditions. The upper plants are undesirable, one having weak stems and the other lacking vigor. The lower plants are more desirable. They differ in density of plant and number of culms. (*Courtesy of Myers.*)

selected at the Minnesota station that were self-pollinated for a 3-year period. One of these strains is from a single self-pollinated line and the other from a combination of several self-pollinated lines. In preliminary comparisons with normal commercial timothy, both strains appear somewhat superior to the commercial variety. These and other results of a similar nature prove that in some cases improved varieties can be obtained directly by the utilization of self-pollinated lines from crops that presumably belong to the cross-pollinated group. In brome grass, *Bromus*

inermis, Kirk (1932) obtained a vigorous nonstooling line after four generations of selfing.

Most perennial grasses show considerable self-sterility, and the question of the desirability of selection in self-fertilized lines as a method of breeding grasses is undecided at the present time. Beddows (1931) has summarized many previous studies on seed setting in the grasses and presented further data comparing seed setting from enclosed inflorescences in relation to seed setting under normal open-pollinated conditions in terms of a ratio of heavy seeds per 100 spikelets in free inflorescences (open-pollinated conditions) over enclosed (self-pollinated conditions). Most annual species of grasses were rather highly self-fertile, whereas many of the perennial species set much less seed on enclosed inflorescences than under normal open pollination. In perennial species of grasses, the ratio of seed setting of free over enclosed, F/E, ranged from 0.95 in *Agropyron tenerum* to 152.95 in *B. inermis*. In *Lolium perenne*, Jenkin (1931*a*) studied several different plants and clonal progeny extensively. Plant 43 produced only an average of 0.8 seeds per 100 spikelets when self-pollinated, whereas plant 48 produced 117.6 seeds. Wholly self-fertile lines were easy to obtain. With orchard grass, *Dactylis glomerata*, Stapledon (1931) obtained some self-fertile and self-vigorous plants, several inbred lines remaining vigorous after 5 years of selfing. On the average, however, the plants resulting from selfing are much less vigorous than those derived from crossing.

With red clover, *Trifolium pratense*, Williams (1931*a*) found 3 plants out of 262 original plants to be truly self-fertile; he explained self- and cross-incompatibility on the basis of an extensive series of self-sterility alleles. White clover, *T. repens*, was highly self-sterile according to Williams (1931*b*), although less so than red clover. Atwood (1940, 1941) has studied self- and cross-incompatibility in *T. repens* and explains his results on the basis of a multiple-allelic series of factors. One plant out of 615, when self-pollinated, set seed freely and may have carried a factor for self-fertility.

A highly self-fertile line of red clover has been bred at the Minnesota station, and in crosses between this line and normal self-sterile plants fertility continues to be the dominant type under self-pollination conditions. Crosses between the self-

fertile line and normal plants were used as a basis for selection under isolated normal open-pollinated conditions. The origin consisted of 50 plants from commercial red clover crossed with the self-fertile line. The plan of selection consisted of growing about 1000 plants each generation in a nursery individually spaced, the selection of 100 vigorous, desirable plants, and the discarding before flowering of other plants in the nursery, allowing cross-pollination of the selected plants. Similar selection was made in another isolated plot using normal commercial northern-grown seed. After three generations of selection, the two types of origin were compared by growing their progeny in rows, with the result that the cross of the self-fertile with normal appeared less vigorous than the selection from normal commercial red clover.

It is generally believed that the cucurbits, comprising cucumbers, muskmelons, watermelons, pumpkins, and squashes, belong to the cross-pollinated group of plants. Rosa (1927) stated that the amount of cross-pollination in melons varied with the variety and ranged from 5 to 73 per cent. Whitaker and Jagger (1937) state that cucumbers, squashes, and pumpkins are normally strictly monoecious, whereas muskmelons and watermelons are andromonoecious. Andromonoecious species bear bisexual or complete flowers instead of strictly pistillate ones, in addition to staminate flowers. When one considers the way that these plants are grown and the necessity of insect pollination, it is apparent that frequent cross-pollination must occur.

The cucurbits, as a group, show less reduction in vigor due to inbreeding than most members of the cross-pollinated group of plants. Bushnell (1922), Haber (1929), and Cummings and Jenkins (1928) found no great loss in vigor as a result of continued self-pollination with squashes. Cummings and Jenkins studied the effects of continued self-pollination for 10 generations. Similar results were obtained by Porter (1933), Rosa (1927), and Scott (1932) with watermelons. C. F. Poole reports (unpublished) that lines of the Northern Sweet watermelon that have been selfed for seven generations, show no reduction in size of melon when compared with the commercial lines of the same variety. Whitaker and Jagger concluded that hybrid vigor probably did not occur in any of the cucurbits. There are, however, two recent reports by Hutchins (1938) with the cucumber and by Curtis (1939) with summer squash that show marked

hybrid vigor. Hutchins suggested that it would be feasible to utilize the hybrid vigor of F_1 crosses in commercial production. Curtis outlined a method for the production of hybrid seed with summer squash, *Cucurbita pepo*, by growing the two varieties to be crossed in alternate rows and the removal of all male flowers from the seed variety before the male flowers have opened.

INHERITANCE OF SELF-INCOMPATIBILITY

Crane and Lawrence (1934) draw a distinction between incompatibility and sterility. Incompatibility is due to some physiological hindrance to fertilization. The pollen and ovules—or at least a good proportion of them—are functional, the failure to obtain seed being due to slow pollen-tube growth. Sterility is classified by Crane and Lawrence into: "(1) generational sterility, due to the failure of any of the processes concerned with the normal alternation of generations, namely, development of pollen, embryo-sac, embryo and endosperm, and the relation of these to one another and their parents regardless of the cross made and (2) morphological sterility due to suppression or abortion of the sex organs."

Many species of plants are often self-sterile, and among these there are many plants of economic importance. These include fruits, perennial grasses, rye, some clovers, alfalfa, sugar beets, some *Brassica* species, and some plants grown for ornamental purposes. East (1929) and Brieger (1930) have given extensive reviews of much of the literature.

Crane and Lawrence (1934) credit Prell (1921) with first suggesting a genetic explanation of self-sterility on the basis of a series of self-sterility alleles and East and coworkers for the first proof of such a series in *Nicotiana*. Self-incompatibility appears to be a somewhat more desirable term than self-sterility for those cases where self-fertilization is prevented, although the pollen grains and egg cells are functional.

There has been a rapid accumulation of information regarding self-incompatibility in recent years, and in many species rather clear demarcation between self-compatibility and self-incompatibility. In other cases, the differences are not so clear-cut, and there may be a gradual graduation from self-fertility to self-sterility through a series of causes. Crane and Lawrence

have given evidence for the conclusion that in some cases this may be the result of polyploidy and the duplication of several series of sterility alleles.

Two types of inheritance seem of general interest. The opposite-factor hypothesis furnished a satisfactory explanation of self- and cross-incompatibility in tobacco by East and coworkers. The genes responsible belong to a series designated by S, and like other alleles, two factors may be carried by a single diploid plant, a series of 15 such allelic factors having been found in tobacco. A pollen tube carrying any one of these alleles, S_1 to S_{15}, shows slow pollen-tube growth in the stylar tissue carrying the same factor but normal pollen-tube growth in stylar tissue carrying a different genetic factor for self-incompatibility. A factor for self-fertility S_f was found, also, that was functional with any of the S_1 to S_{15} alleles, and self-fertility was dominant to sterility in crosses. Self-fertility of this nature would breed out incompatibility in the selfed progeny of crosses, which would make it possible to add the factor for fertility if desired.

Types of results that may be expected will be illustrated briefly. Parental genotypes F_1, F_2 and backcross progeny in a diploid organism make clear the types of breeding behavior. Two self-sterile plants are used as parents in the hypothetical illustrations, their genotypes being S_1S_3 and S_2S_4. When S_1S_3 is self-pollinated, seed production does not commonly result, since pollen tubes carrying either of the alleles S_1 or S_3 grow too slowly in stylar tissue of the same genotype. Exceptions to this rule have occurred in many species of self-sterile plants leading to homozygous individuals of the genotype S_1S_1 and S_3S_3, but seed is too infrequent to make this method of seed production efficient as a means of controlled selection in self-pollinated lines of self-sterile species. With some species such as cabbage, *Brassica oleraceae*, controlled bud pollination of a self-sterile plant gives good seed production, and selection in self-sterile lines may be practiced, leading to the isolation of homozygous lines. A cross of $S_1S_3 \times S_2S_4$ produces four types of F_1 offspring S_1S_2, S_1S_4, S_2S_3, S_3S_4. Each of the types is self-sterile but fertile with their parents in backcrosses and with each other. A cross of $S_1S_2 \times S_1S_4$, for example, will produce plants that are S_1S_4 and S_2S_4. The reciprocal cross of $S_1S_4 \times S_1S_3$ will produce S_1S_3 and S_3S_4.

When the self-fertility allele S_f is present, continued self-pollination leads to the rapid isolation of self-fertile genotypes. For example, $S_fS_f \times S_1S_3 \rightarrow S_fS_1$ and S_fS_3. If S_fS_1 is self-pollinated, only two genotypes result, one like the parent S_fS_1,

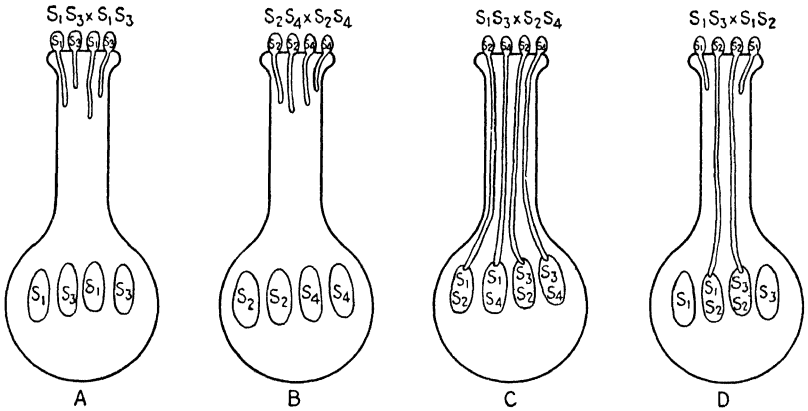


FIG. 35.—Diagrammatic representation of pollen-tube growth in compatible and incompatible crosses. (*a*) and (*b*) Incompatible, slow pollen-tube growth; (*c*) compatible, all pollen able to effect fertilization; (*d*) only S_2 pollen functional.

the other homozygous for S_fS_f. The pollen tube developing from a pollen grain carrying S_1 makes slow growth in stylar tissue (S_fS_1) carrying the self-sterility allele S_1.

Riley (1934, 1936) has explained self-sterility in *Capsella grandiflora*, a diploid species with eight haploid chromosomes, on the basis of the sporophytic nature of the parent plants and on the interaction of two pairs of genes. Before giving the genetic explanation, a brief summary of the results of the crosses will be given.

Three intrasterile, interfertile classes have been found in *C. grandiflora*. These have been designated classes *A*, *C*, and *B*. Class *A* × class *C* will produce classes *A* and *C*, or *A*, *B*, and *C*, but never *A* and *B* only. Class *A* × class *B* will produce classes *A* and *B*, or classes *A*, *B*, and *C* or classes *A* and *C*. Class *B* × class *C* will produce classes *B* and *C*, or class *C* only, but never class *A*. Reciprocal crosses give the same result.

When a plant of *C. grandiflora* was crossed with any one of the three self-fertile species of *Capsella*, the F_1 was fully fertile and completely self-compatible. Segregation for self-fertility and self-incompatibility occurred in the F_2.

The following table (Riley 1936) gives the genetic explanation of results from crosses within and between the three self-incompatible groups.

TABLE 30.—STERILITY AND FERTILITY IN CROSSES WITHIN AND BETWEEN THE THREE SELF-INCOMPATIBLE GROUPS OF *C. grandiflora*

Genotype	Class *A*		Class *C*			Class *B*
	TtS^cS^c	TtS^cs	$Ttss$	ttS^cS^c	ttS^cs	$ttss$
TtS^cS^c	*S*	*S*	*S*	*F*	*F*	*F*
TtS^cs	*S*	*S*	*S*	*F*	*F*	*F*
$Ttss$	*S*	*S*	*S*	*F*	*F*	*F*
ttS^cS^c	*F*	*F*	*F*	*S*	*S*	*F*
ttS^cs	*F*	*F*	*F*	*S*	*S*	*F*
$ttss$	*F*	*F*	*F*	*F*	*F*	*S*

S = sterile, *F* = fertile.

These results were explained on the basis of the sporophytic nature of the parent plants. Members of class *A* were incompatible in crosses together, because they possess the dominant gene *T*, which is epistatic to S^c. Any plant that is homozygous for the recessive condition, or *tt*, is fertile in reciprocal crosses with members of class *A*. Factor *T* is never in the homozygous condition, since plants bearing it can be crossed only with the homozygous recessive *tt*.

A second pair of genes S^cs is responsible for the differentiation of classes *C* and *B*. Class *C* carries this pair of genes either in the homozygous condition S^cS^c or the heterozygous condition S^cs, and class *B* is homozygous for the recessive condition *ss*.

A self-compatible factor S^f is a member of the *S* series and dominant to S^c or *s* and epistatic to *T*, and S^c and *s* are hypostatic to *T*.

East (1934) suggested that substances in the stylar tissue of *Nicotiana* react with substances in the pollen tube to slow up the rate of the pollen tube growth in self-incompatible combinations. The further suggestion was made that, in most plants, these substances are not present in the young bud but appear during the 24 hr. preceding the opening of the flower. In self-sterile genotypes that are self-fertile when pollinated 24 to 48 hr. before the flowers open it was presumed that these substances were not

produced during the 24 hr. preceding flower opening. In another type in which self-sterile plants are self-fertile at the end of the flowering period it was presumed that these plants were unable to produce an adequate amount of the inhibiting substance late in the flowering period. The inhibitory effect appears to be localized in a certain region of the style, since the growth rate of the pollen tube is slowed up markedly as the pollen tubes reach this place, but after this region is passed the growth rate again approaches normal. In *Capsella*, the pollen grains in self-incompatible matings do not germinate, or produce tiny abortive tubes only. This has led Riley to suggest that these inhibitory substances are located at the very end of the stigma in the stigmatic hairs. Yasuda (1934) believes these inhibitory substances originate in the ovule in petunia, from which they may ascend the stigma, depending on genetic differences in the plants and the environmental conditions under which they are grown. If they reach the stigma, they may inhibit germination of the pollen grains. If they only reach the style, they may inhibit pollen-tube growth in the stylar region, and in some cases these substances stay in the ovary and inhibit pollen-tube growth in the ovary. The suggestion is made also that weak self-fertility may be the result of a low production of these inhibitory substances.

Studies in Wisconsin by Brink and Cooper (1939) and Cooper and Brink (1940) with alfalfa deal with partial self-incompatibility, the type of self-sterility that probably occurs very commonly in many crop plants. Cross-pollination produced a much higher average number of seeds per flower than self-pollination. In a comparison of seven plants selfed, with crosses between them, 14.6 per cent of self-pollinated ovules were fertile, whereas 66.2 per cent of cross-pollinations led to fertilization. A low degree of fertilization under conditions of self-pollination was believed to be explained primarily on the basis of the oppositional-factor hypothesis.

Of the ovules that became fertile, 34.4 per cent containing inbred embryos and endosperms collapsed within 6 days after fertilization; in the cross-pollinated plants, only 7.1 per cent, containing hybrid endosperms and embryos, collapsed. These differences are highly significant and seem to be dependent upon the relative rate of growth of the endosperm tissue and embryo.

The collapse of ovules during the early-development stages after fertilization has been called somatoplastic sterility by Brink and Cooper. They say:

> The embryo sac in the mature ovule of alfalfa is surrounded by two integuments. The inner integument, which is composed of two layers of cells, lies in direct contact with the embryo sac except at the chalazal end where a few disintegrating cells, remnants of the nucellus, are found. Shortly after fertilization active cell division is initiated in the integuments as well as in the endosperm mother cell and the zygote.
>
> The critical factor for survival seems to be the manner in which the translocated food is shared between the endosperm, on the one hand, and the inner integument, on the other. The partition of nutrients appears to depend upon the rate of growth inside and outside the embryo sac.

The endosperm is considered to be the dominant tissue within the embryo sac. When the endosperm keeps pace in its growth with the surrounding material, tissue development of the seed continues in a normal manner. The rate of growth of the embryo is much slower than that of the endosperm and not very different in hybrid than in inbred embryos. The writers say, "The initial conditions in the ovule outside the embryo sac being alike in the two cases, it seems clear that the higher survival following crossing is the result of the more active growth of the hybrid endosperm. Conversely, following self-fertilization, the rate of growth of the endosperm is frequently so low that the balance soon shifts in favor of the integuments."

Other cases were noted by Brink and Cooper from species crosses, where fertilization had taken place but early collapse after fertilization followed because of slow growth of the endosperm and the lack of nutrients for the growing embryo. Although these results have not been placed directly on a genetic basis, it seems probable that they result from causes similar to those responsible for the reduction of vigor in selfed lines of cross-pollinated plants. If one accepts the Mendelian explanation of heterosis, then it seems probable that the early collapse of ovules after fertilization is due primarily to genetic causes.

Other cases of self-sterility are known. Heterostylism, *i.e.*, differences in relative length of the styles and stamens, may cause a lack of seed production under conditions of self-pollination.

Proterandry or proterogyny also may be causes of cross-pollination and make self-fertilization difficult.

Although much is known regarding problems of self-sterility, different investigators have reached widely different conclusions regarding the possibilities of using controlled self-pollination as an aid in breeding. These differences in opinion are doubtless dependent upon species and varietal differences in response to self-pollination, or the effects of differences in environmental conditions. The extent to which controlled self-pollination can be used in breeding remains an unanswered question in many cases.

METHODS OF BREEDING

Certain principles rather generally accepted as of importance in relation to the controlled method of breeding in corn appear to be applicable to other cross-pollinated crops. Certain of these may be restated.

1. Yield and many other characters of economic importance are the end result of the interaction of multiple factors.

2. Inbred lines show wide genetic differences and, as a rule, are less vigorous than the normally pollinated varieties from which they originated.

3. Inbred lines differ in combining ability in crosses. Although there is considerable evidence that combining ability in crosses is positively and significantly correlated with those characters that are expressions of vigor in the inbred, it is equally evident that, of two inbred lines that in themselves seem equally desirable, one may give much greater vigor than the other, on the average, in crosses with unrelated inbreds.

4. Combining ability of an inbred may be tested by crossing it with a commercial variety, *i.e.*, the top-cross is a relatively satisfactory method of learning relative combining ability. Crosses with a series of inbreds used as testers is another method now in use by the corn breeder for testing combining ability. Some investigators have advocated the use of testers that in themselves are undesirable for important characters.

5. Combining ability is inherited in much the same manner as other quantitative characters. If two low combining lines are crossed and selection practiced during the segregating generation under controlled self-pollination until relative homozygosis is

obtained, most of the resulting inbreds will be low in combining ability. Conversely, inbreds selected from a cross of high-combining inbreds are mostly high in combining ability.

6. Genetic diversity is of importance in relation to heterosis. Crosses between inbreds from a different origin show greater heterosis, on the average, than from a related origin.

7. Combining ability may be determined during the early generations of selfing by means of the crossing test.

When controlled cross-pollination can be carried out on an extensive scale at a reasonable cost, it seems that the method of breeding by controlled pollination that has been developed for corn can be applied directly to other plants of the cross-pollinated group. In some cases, it seems feasible to introduce a factor for male sterility in one of two inbred lines that are to be used to produce F_1 crossed seed for commercial seed production. The two lines to be crossed would be interplanted, and all male fertile plants would be removed before pollination. With perennial plants, the same field could be used for several years. Further increases for seed production could be made by vegetative propagation.

A method suggested by Pearson (1932) for breeding cabbage is of interest where self-incompatibility is of common occurrence and controlled bud pollination leads to the production of a considerable amount of selfed seed. In cabbages, both self-fertile and self-incompatible lines may be obtained. Pearson suggests the selection and isolation of self-incompatible lines by pollinating in the bud stage. These self-incompatible lines can be differentiated from the self-fertile by pollinating at anthesis also and discarding the lines that are self-fertile, *i.e.*, lines that set seed when pollinated at anthesis. After a considerable number of self-incompatible lines have been selected, these then may be tested for combining ability. Although not suggested by Pearson, it seems that the inbred-variety cross method would be desirable to use in the first elimination of lines, with the use in the crossing study of only those lines that proved to be good combiners. After selecting the best inbred lines by this means, artificial crosses between lines should be made, making the crosses at anthesis. Those crosses that set seed would then be tested for producing ability. After obtaining a desirable cross, it could be maintained by bud pollination of the parent

lines and the continued production of controlled cross-pollinated seed by interplanting members of the two lines for seed production.

A plan of breeding grasses originally adopted at Aberystwyth, Wales, and in New Zealand (Levy 1933) is of general interest. It consists of collecting material from its natural habitat, from foreign sources, and from other breeders, and of making a study of several thousand individual plants, perhaps with the final selection of not more than 100 or 200 out of 5000. The progeny of these selected plants may be increased and given further study, with the use of one of several methods of breeding, depending on the nature of the material, the possibility of self-fertilization, and the extent to which it is possible to isolate vigorous self-fertile lines. In many cases, a rapid increase of material from the selected plants may be desirable. A simple method consists of interplanting the clonal progeny of these selected plants, allowing them to cross by natural means. When facilities are available for an intensive breeding program with a particular species, desirable-appearing clonal lines or closely bred lines that may or may not have been previously bred by controlled self-pollination may be used as a basis for breeding of improved varieties. By means of brother-sister mating or diallel crossing and the test of crosses in F_1 and in F_2, the more desirable progenies may be isolated and combined to produce improved synthetic varieties.

Some investigators are using the so-called Macauley (1928) method as a means of isolating relatively homozygous lines, where controlled self-pollination, because of self-sterility, or where reduction in yield as a result of continued selfing is so great that the isolation of selfed lines does not seem desirable. Macauley suggested a method of close breeding for corn that may be adapted for this purpose. As applied to corn, it consists of growing the progeny of selected ears each in an individual plant plot of approximately 200 plants, preventing cross-pollination between plots by means of natural barriers such as the use of border rows of a much later maturing corn and thus forcing pollination within each plot. The more desirable plots are selected each generation and several ears again selected to plant the isolated plots for the next generation. It was concluded on theoretical bases that four or five generations of this sort of selection would be equiva-

lent, in an approach to homozygosity, to a single generation of selfing. The inbred lines obtained by this method of breeding, where sufficient vigor is retained, could be used directly as an improved variety, or several lines could be combined to produce a synthetic variety.

When intensive studies of improvement have not been made previously, the recognition and propagation of improved ecotypes that have developed through natural selection may prove of value. By these methods, a considerable series of new strains of great value have been developed in New Zealand. These include Hawks Bay and Poverty Bay perennial rye grass, Akaroa cocksfoot (orchard grass), New Zealand white and New Zealand extra-late red clover, Marlborough lucern, and New Zealand brown top.

In spite of some of the difficulties, it seems advantageous to outline methods of breeding. These are based to a considerable extent on methods found applicable to corn.

Outline for Improvement of Cross-pollinated Plants by Controlled Pollination Methods

I. Selection in self-pollinated lines.

A. In general, use adapted varieties, and artificially self-pollinate as many plants as can be handled with the available facilities; unadapted varieties may be used if any desirable character is wanted.

B. Grow the progeny of each self-pollinated plant from self-fertilized seed. The number of plants in each selfed line of the first and succeeding generations should be sufficient to give an adequate sample of the progeny. This number ordinarily should not be less than 20, and a minimum of 30 to 40 plants is desirable. In many crops it will be advantageous to start the seedlings in the greenhouse and transplant into the fields. The plants should be spaced far enough apart to permit individual study.

C. Self-pollinate one or more desirable-appearing plants from each desirable first-generation selfed line. In general, plants of at least average vigor should be selected for selfing.

D. Following the procedure in *C*, grow successive generations of selfed lines until relative uniformity is secured. As the process of selection proceeds from first to later generations, greater weight should be given to vigor of growth and more emphasis placed on high fertility. As elimination of weak and undesirable lines is effected, their place in the nursery may be filled with additional selections from the strong desirable lines, with new first-generation selfed lines, or by selections from crosses between selfed lines.

E. Any selfed lines of promise may be tested for reaction to disease in a special disease garden, or a disease epiphytotic may be induced in the self-fertilization plots.

II. Improvement of selfed lines.

A. Backcrossing. A desirable method when one wishes to retain all or nearly all the characteristics of one line and add some characters to it. Easy of accomplishment when the character to be added is inherited in a relatively simple manner.

Examples from corn:

1. To add yellow endosperm to a selfed line that is desirable in other characteristics and is breeding true for white endosperm.
2. To add tender pericarp to a sweet-corn variety or selfed line that has tough pericarp.

B. Convergent improvement. A desirable method of increasing the vigor of each of two desirable selfed lines that combine well in an F_1 hybrid without modifying their combining ability.

C. The pedigree method. Select selfed lines as parents that have complementary characters, *i.e.*, lines that excel in different desirable characters. After making the cross, selection during several generations of selfing is practiced until practical homozygosity is reached. *Example:* One parent with strong stalk, *i.e.*, ability to withstand lodging, the other with good general vigor but weak stalk. Make the cross, and self-pollinate, and select during the segregating generations.

III. Use of selfed lines as breeding material.

A. 1. Top-crosses or inbred sire crosses, an inbred line crossed with a variety.

a. Of value in some cases as a commercial hybrid, or as a basis for the selection of an improved clon in such crops as potatoes.

b. A desirable method of testing the combining ability of selfed lines. By this means, the more promising lines are selected to test in single, three-way, double crosses or in the production of a synthetic variety.

2. Selfed lines, if sufficiently vigorous, may be increased for use as a new variety.
3. Single crosses between two selfed lines may be made and the cross grown as the commercial crop, providing the selfed lines are sufficiently good seed producers.
4. Double crosses between two single-crossed hybrids may be made and the cross grown as the commercial crop. Advanced-generation single crosses may be used, particularly as the male parent.
5. Three-way crosses may be used as the commercial crop. An F_1 cross of two selfed lines may be used as the female and a selfed line as the male parent.
6. New varieties may be synthesized by composite crossing of several inbred lines or in special cases from two lines.

B. Compare new varieties and F_1 hybrids with standard varieties by means of replicated field-plot trials for a sufficient length of time to establish their value.

C. Production of seed of F_1 hybrids and new varieties by increasing seed of improved varieties and inbred lines in isolated plots.

IV. Crop plants in which self-sterility is a factor.

A. Selection of self-fertile lines and the addition of the factor for fertility to many lines of the crop plant, *i.e.*, the breeding of self-fertile lines and their use later as F_1 hybrids or their combination into synthetic varieties.

B. Self-pollination for a generation or two until some desirable character is homozygous, followed by the combination of several lines.

C. Selection in self-sterile lines, pseudofertile, by pollination in the bud stage or other means such as the self-pollination of many flowers and the use of the few seeds obtained as a means of selection in normally self-sterile lines.

D. Cross-mating of plants selected on the basis of outstanding characteristics. Progeny of crosses selected and better types isolated.

1. Strain building on the basis of selection of several plants as parents and their bulk crossing by natural means. A broad system of mass selection is practiced. This method is applicable to the rapid increase of ecotypes that have developed by natural selection under a particular set of environmental conditions.
2. Strain building by brother-sister mating.
3. Diallel crossing by hand. New strain developed from several of the better crosses.

In diallel crossing, the breeder will have available inbred, closely bred, or clonal lines that have outstanding characters. After the crosses have been made, it may be desirable to test their progeny in F_1 and F_2. In some cases grazing trials or other tests of F_2 crosses will be helpful. Crosses will be combined in a synthetic variety from parent plants or lines that combine well with other lines to be used in the synthetic variety. It will be possible, in many cases, to use F_1 crosses to make the first combination of selected lines for use in the improved variety.

CHAPTER XVII

SEED PRODUCTION

The breeding of improved varieties of crop plants is carried on, as a rule, by specialists who are trained in plant-breeding methods and who have a knowledge of the needs of the grower and consumer. Although a seed producer may undertake the problem of breeding in some cases, the primary task of the seedsman will be to produce high-quality seed of varieties and strains of known value.

Good seed of any farm crop must be produced from a variety or strain that is superior, insofar as that is possible, in the following respects:

1. Adaptability to the locality and soil.
2. Purity of type.
3. Yielding ability.
4. Desirable agronomic characters.
5. Disease and insect resistance.
6. Quality for particular characters.

The seed of this adapted variety must be superior in the following characters:

1. Germinating ability.
2. Color of seed and seed weight.
3. Uniformity.
4. Freedom from seed-borne diseases.
5. Freedom from noxious and other weeds.
6. Freedom from other damage.
7. Freedom from mixtures with other varieties.

These characteristics of good seed are, in general, appreciated by seed growers. The first step in the production of good seed is the selection of the variety or varieties to be grown.

SELECTING THE VARIETY

Improved varieties of farm crops bred by investigators at federal or state agricultural experiment stations, including varieties of wheat, oats, barley, and cotton, are registered through

a cooperative agreement by the Bureau of Plant Industry of the U.S. Department of Agriculture and the American Society of Agronomy. Registration is under the direction of a committee of the agronomy society and is based on information from yield trials, carried on for at least 3 years, in comparison with standard varieties at federal or state agricultural experiment stations. To be eligible for registration, a variety must be significantly superior to the standard in some important character or characters and equal in other important characters. Registration consists of giving the new variety a register number and publishing a description of its origin and characteristics in the *Journal of the American Society of Agronomy*. Plant and seed samples are furnished by the person or institution submitting the request for registration.

Many of the state agricultural experiment stations list recommended varieties and describe the conditions under which the varieties usually give the most satisfactory performance. These lists are based on actual field trials conducted on experiment-station or farmers' fields in comparison with standard varieties. The *University of Minnesota Agricultural Extension Folder* 22, 1941, revised whenever it seems necessary, gives the general principles used in Minnesota in drawing up the recommendations. Somewhat similar methods are used in other states. A variety is added or removed from the recommended list of the Minnesota Agricultural Experiment Station by vote at an agronomy conference held each year. The following statement is quoted from the folder.

> The list of recommended varieties for Minnesota has the joint approval of agronomists, plant breeders, and plant pathologists of the central experiment station at St. Paul and of the superintendents and agronomists of the various branch stations at Waseca, Morris, Crookston, Grand Rapids, and Duluth. A variety must have been tested in experimental plots for at least three years to be eligible for recommendation. The basis of recommendation is satisfactory performance in competitive trials when compared with standard varieties. These tests are conducted at the central and branch stations, in cooperative trials on farms, and, in addition, comparative trials of reaction to disease are conducted in specially prepared disease nurseries at the central station. Varieties introduced from outside the state are given the same careful trial as those developed in Minnesota.

The list is followed by a statement of the important characters of each recommended variety and its origin and regional adaptation. A brief statement of varieties that are not recommended is also given.

In Canada, the Canadian Seed Growers' Association has accepted responsibility for deciding which varieties shall be eligible for the production of certified or registered seed. In general, varieties are accepted on the basis of their performance in adequately conducted field trials in comparison with standard varieties. The list of varieties eligible for use in the production of registered seed is an important means in Canada of selecting varieties for particular conditions.

Many of the states in the United States have crop-improvement societies composed of growers interested in problems of seed production. In some cases, the state association may select varieties eligible for seed certification. These varieties are chiefly those recommended by the state experiment stations, although in some cases a few varieties in addition are selected by the varietal committee of the crop-improvement society.

Large and small seed companies may in some cases breed or select an improved variety. Improved varieties are described in seed catalogues, which helps to make the characters of varieties known to the general public. Many of the corn hybrids used in the corn belt are produced and introduced by seed companies. These companies use inbred lines of their own breeding together with those released by federal or state workers and introduce and sell seed of hybrids for commercial growing under their own pedigree, which in many cases is kept secret, although the pedigree must be filed with a state official to comply with certain state laws. In other states, all that is required, in addition to the usual information required by seed laws, is a statement of the type of hybrid and average days required to mature the hybrid in various sections of the state.

Extensive yield trials of commercial seed-company hybrids, in comparison with federal and state-experiment-station hybrids, are made annually by most of the corn-belt experiment stations. These trials are under the supervision of the Agricultural Extension Division and the State Agricultural Experiment Station. An entrance fee is charged for each commercial hybrid grown in the trials. Reports of the results of these trials are used by

growers as a means of selecting the hybrid that is best adapted to their conditions.

The value of new varieties and their characteristics are brought to the attention of growers by holding field days at the various experiment-station fields at or just prior to harvest time, when yield trials are discussed and the characters of particular varieties may be observed by the grower.

In spite of the various methods used to inform the grower of the relative merits of different varieties a large amount of seed of overexploited and unadapted varieties is sold annually by seedsmen. The loss could be done away with by a greater effort to inform the producer of varietal characteristics and by a wider use by the farmer of the information now available in the hands of the state agricultural colleges, the experiment stations, and the agricultural extension service.

FIRST INCREASE OF SEED OF A NEW VARIETY

A large proportion of new varieties of farm crops in the United States are bred by state or federal investigators at agricultural experiment stations. After deciding to recommend a new variety, the problem of increase of seed and introduction of the variety becomes of major importance. Many of the state experiment stations keep on hand a small amount of pure seed of all recommended varieties that serves as an initial source of pure seed supply. First increases of new varieties of crop plants often are made on experiment-station fields. Subsequent increase is usually in the hands of seed growers who are members of the state crop-improvement association.

After making the initial increase of seed of a new variety at the state experiment station, further increase is made in Minnesota by the so-called "approved grower plan." A committee of agricultural experiment-station workers decides how much of the available seed supply will be distributed in each county. Growers are selected by a county committee consisting of the extension agronomist, county agent, and three farmers appointed by the president of the Minnesota Crop Improvement Association. The "approved grower" of registered seed is one who has the following qualifications:

1. Willingness to cooperate to the fullest extent with the experiment station, extension service, and other agencies interested in pure seed production.

2. Available clean land for seed production.
3. Facilities for storing seed so that mixtures may be avoided.
4. Previous satisfactory record in crop-improvement work and in the community of which he is a member.

These approved growers purchase seed from the experiment station and agree to place at the disposal of the experiment station, if requested, at a price agreed upon by the seed-distribution committee, all seed over and above that agreed upon for use on his own farm. After this initial increase in the hands of approved growers, subsequent seed increase is made by other seed producers of the Minnesota Crop Improvement Association or by others interested in seed production.

It was emphasized in the chapter on Corn Breeding that three-way and double crosses in field corn were used chiefly by the commercial corn grower. The propagation of inbred lines, single crosses, and the production of three-way or double crosses by the seed grower are essential phases of seed-corn production. The larger seed companies take care of the initial increase and maintenance of purity of the inbred lines used in their own pedigrees.

There are two rather distinct plans that are followed for hybrids released by state or federal workers. One method consists of sales of small quantities of pure seed of inbred lines to seed producers who make the subsequent increase of the inbreds and single crosses necessary for three-way or double-cross seed production. Some private breeders specialize in producing seed of single crosses. All state experiment stations in the corn belt have adopted a plan of initial seed increase of inbreds and single crosses for newly released hybrids. Most of the experiment stations release the inbreds after 2 or 3 years have elapsed since the new hybrid was first released. Minnesota and Wisconsin have developed methods for the increase of inbreds and first crosses in sufficient quantity for the needs of all corn-seed producers in their respective states. Similar work is carried out in Ohio by a cooperative organization of seed growers. Inbreds are not released for the hybrids that have been bred and recommended by station workers in Wisconsin and Minnesota.

The Minnesota studies have led to the conclusion that considerable care must be taken to maintain the purity of inbred lines. The methods used and some of the conclusions reached

have been summarized by Borgeson and Hayes (1941). The plan now used is outlined as given by these writers.

Hand-crossed and selfed seed of all inbred lines needed in the corn program is planted each year in foundation plots at both the Southeast and Central stations. The crop risk is distributed as much as possible by planting at two stations with several dates of planting at each location. Sufficient selfed ears are produced to provide the necessary seed that is needed the following year in the crossing plots where single crosses are produced. The selfed ears are inspected both before and after drying.

The seed is harvested and dried in fine-meshed bags in tray driers. Twenty to 30 individual representative selfed ears of each culture are saved and the balance of the selfed seed bulked to use for producing single crosses. Short "ear-to-row" cultures from 20 to 30 selfed ears of each inbred are planted also in the foundation plot. Hand crosses are made between the individual ear cultures obtaining several crossed ears from each combination of "ear-to-row" cultures as follows: 1×2, 2×3, 3×4, etc., where 1 to 4, etc., represent the "ear-to-row" cultures of each of the inbred lines, respectively. The hand-crossed ears in each culture are examined and desirable crosses are bulked using representative cultures. The crossed bulked seed is used the following year as the parental source for the rather extensive hand selfing program that furnishes the major source of selfed seed for single cross increases. In some seasons it is necessary to use hand controlled sib-pollination when self pollination for any reason in some lines does not prove feasible. The plan then consists of alternately producing selfed and crossed seed for each inbred line.

The major features of the plan may be summarized briefly as follows: When an inbred line seems relatively homozygous, sufficient selfed seed of each inbred is produced each year to plant the necessary single cross plots the following year. The seed planted for the selfing plot is obtained the preceding year from hand-pollinated crosses made by crossing the progeny of "ear-to-row" cultures within the inbred lines produced from selfed ears.

The rapid increase in demand for hybrid parent stocks made it necessary to produce the larger part of the single crosses with individual farmers. Two types of contracts have been used, one calling for an acre rental fee and the other on the basis of production usually by pound units. On the acre basis it did not appear that growers were sufficiently interested in all cases to place the work on a desirable basis. There was no great incentive to produce a high yield. On the other hand, the yields of the single crosses were unpredictable, and it was difficult to arrive at a satisfactory price on the pound basis.

This year a new form of contract has been used with the majority of the growers. The grower is permitted to retain a share of the seed stocks produced for his part in the contract. The balance of the seed is then turned over to the experiment station for sale to other growers. Small plots used for the increase of advanced generation seed are contracted on the acre rental or unit payment plan.

During the past season it was possible to examine the first results of the new methods of seed increase. All the single crossing plots were planted with hand-pollinated seed. The purity of the parent lines was highly satisfactory. Actual counts showed the percentage of off-type plants to run on an average of about 0.25%, or 1 to 400. Any rogues present were removed prior to tasseling.

From previous experience it is believed that it will be necessary to provide hand-pollinated seed every year for the single cross plots. From indications to date, the methods of increase outlined in this article should provide both the purity and quantity of inbred seed desired.

SEED CERTIFICATION AND REGISTRATION

The Canadian Seed Growers' Association.—The Canadian Seed Growers' Association, first organized early in the present century, was modeled after the Swedish Seed Association that was first established in 1886. The objects of the Canadian association, which was incorporated in 1920, may be made clear by quoting from its Letters Patent, as given by Wiener (1937).

(*a*) Advancing the interest of Canadian agriculture by encouraging seed growers and farmer members to maintain a high standard of excellence.

(*b*) Developing the standards of quality for varieties and strains that shall be eligible for registration.

(*c*) Establishing and maintaining a record of these varieties and strains that are approved for registration.

(*d*) Fixing standards for the different classes of propagating stock of varieties and strains that may be eligible for registration.

(*e*) Making provision for the necessary inspection of field crops and propagating stock.

(*f*) Maintaining records of registered propagating stocks produced by members.

(*g*) Encouraging the development and introduction of superior varieties and strains.

(*h*) Providing for the multiplication and dissemination of propagating stock of new varieties approved for registration.

(*i*) Co-ordinating the endeavors of plant breeders and seed grower members of the association with the endeavors of crop producers in general.

(*j*) Utilizing propaganda, advertisement and any other legitimate means to increase the use of registered propagating stock.

(*k*) Developing a home market and if necessary an export market for the disposal of surplus stocks.

(*l*) Such other means as may be found expedient from time to time.

The functions include the selection of varieties that are eligible for the production of registered seed, the production of foundation or elite stock seed of these varieties, the production by individual members of registered seed for sale to commercial growers, such seed having been sealed as registered seed after field and bin inspection.

The work of the association is made possible by an appropriation of the Dominion government, donations from companies interested in high-quality productions, and through the help of Dominion and Provincial organizations interested and engaged in various phases of the crop-improvment projects.

The methods developed in Canada have been used rather extensively as a basis for seed certification in various states in the United States. This work has been carried out through the various state organizations. Uniformity in methods has been developed through the International Crop Improvement Association.

The International Crop Improvement Association.—This association was organized in 1919 at a meeting in Chicago of representatives of the Canadian Seed Growers' Association and of state crop-improvement associations in the United States.

The object of the association can be adequately understood by quoting from the constitution, where the purpose is stated to be

. . . to promote the agricultural interests of the various states and provinces of America, emphasizing especially the improvement of field crops in general and seed improvement in particular by:

(*a*) Encouraging the breeding and improvement of field crops and seeds.

(*b*) Husbanding, propagating, and disseminating Elite, Registered, Certified and Improved seeds.

(*c*) Creating a more active interest in better seeds through circulars, reports, and other publicity, as well as encouraging local, state and international shows.

(*d*) Assisting in the standardization of the seed improvement work being done by member organizations.

The active membership may consist of any national, state, or provincial organization carrying on activities in the interest of improvement of field crops and seeds. At present, between 30 and 35 state crop-improvement associations in the United States and two Canadian associations belong to the international association. This association has held annual meetings since 1919. Through the work of its various committees, standards for seed certification and registration have been developed and applied in the same general manner by the various certification agencies. The annual meetings of workers interested primarily in seed certification and registration have aided in the development of uniform terminology and in the improvement of methods of seed certification.

Description of Seed Classes.—There are three general classes of seed that are recognized by state associations and by the Canadian Seed Growers' Association, although the terminology, as used, varies some from one association to another. The development of more uniform terminology is desirable. The following definitions serve to point out three types of seed.

1. Foundation stock seed is seed that has descended from a selection of recorded origin, under the direct control of the original breeder or of a delegated representative of the state crop improvement association or that is under the control of a state or federal agricultural experiment station. In many states, such seed is registered by the seed-certification official of the state crop-improvement association as "foundation-stock seed."

2. Registered seed is seed of a variety or strain that is the multiplied progeny of foundation-stock seed and that traces directly to it. Both registered and foundation-stock seed must comply with standards of purity and quality laid down by the state crop-improvement association or other certifying agency.

3. Certified seed is seed of a variety or strain recommended by the state agricultural experiment station that has certain required standards of purity and quality. In certain cases, certified seed may not meet all the standards required for registered seed.

At least two inspections are necessary in the production of registered seed, a field inspection during the growing season to check up on purity of type, admixtures of other varieties or other crops, freedom from noxious weeds, etc. If the crop passes this inspection, a laboratory analysis of a representative sample of

seed is made after harvest and after the seed has been processed. This inspection often is carried out through the help of the official state seed laboratory.

The Minnesota Plan for Certain Crops.—As an example of methods, a brief description is given for seed registration of farm crops in Minnesota. Two classes of registered seed are produced by members of the Minnesota Crop Improvement Association called Registered No. 1 and Registered No. 2. A grower produces registered seed by the plan outlined as follows:

1. Plant registered seed. Obtain seed that has passed the association requirements and that comes labeled with the blue or red tag. Only varieties recommended by the Minnesota Experiment Station or the board of directors of the Minnesota Crop Improvement Association are eligible.

2. Plant this seed on clean, well-prepared ground. In the case of cross-pollinated crops, the field should be 40 rods removed from any other crops of the same kind.

3. Apply for field inspection before June 15 on crops other than open-pollinated corn and alfalfa. For these crops, apply before August 1.

4. Return the application blank, completely filled out, that was sent you from the office, along with the dues. Where seed was purchased, also send labels that came with the seed.

After the field and laboratory inspection has been completed, the certifying official issues blue or red tags for Registered No. 1 or No. 2 seed or rejects the seed when the quality is not up to the standards.

The requirements in order to pass the field inspection are summarized in the following statements:

1. Fields will be rejected if plants of field bindweed, leafy spurge, or other noxious weeds, the seeds of which are extremely difficult of separation, are found in the field. If the noxious-weed seeds can be easily separated from the seed crop, the presence of plants of these noxious weeds will not be sufficient cause for rejection of the field.

2. More than a mere trace of other crop plants or plants of other varieties will be cause for rejection of the field. Mixtures that may cause rejection include (*a*) sweet clover in alfalfa, (*b*) durum wheat in spring wheat, (*c*) winter wheat in winter rye, or vice versa, and (*d*) timothy in alsike clover.

3. Instructions are given at the time of inspection regarding roguing, harvesting, and other matters pertaining to the handling of the seed crop.

For hybrid corn, certain isolation requirements are necessary. As a general practice, the seed plot should be 40 rods from other corn. This isolation may be provided by natural barriers, actual distance, male border rows, or a combination of these methods. If natural barriers are used, permission must be obtained in writing from the registration official before planting, in addition to the regular field inspection. Border rows of the male parent may be used to reduce the actual distance required if the corn interfering with the isolation of the hybrid plot is of the same color as the female parent.

The following applies only to detasseling plots of 5 acres or less:

Number of Border Rows of Male Parent	Actual Distance Female Parent Must Be Removed from Other Corn, Rods
0	40
1	37½
2	35
3	32½
4	30
5	27½
6	25
7	22½
8	20
9	17½
10	15
11	12½
12	10

This minimum distance of 10 rods may be reduced on larger fields according to the following plan, provided corn of another color is not involved:

Male border rows needed	Actual distance female parent is removed from other corn, rods	Size of field, acres
13	9	10
13	8	15
14	7	20
14	6	25
15	5	30
15	4	35
16	3	40

The plot shall be detasseled according to instructions furnished for the production of the hybrid. Three inspections during the detasseling period will be made to determine if the work is satisfactorily carried out.

Laboratory requirements for Registered No. 1 and Registered No. 2, the two grades of seed recognized by the Minnesota Crop Improvement Association, are given in Table 31 for small grains, alfalfa, and hybrid corn.

TABLE 31.—REQUIREMENTS FOR REGISTERED NO. 1 AND REGISTERED NO. 2 SEED FOR SMALL GRAINS, ALFALFA, AND HYBRID CORN

Grade	Tag	Laboratory purity, per cent	Maximum allowance, weed seeds			Maximum allowance, crop seeds, per cent	Inert matter, per cent	Germination, per cent
			Noxious*	Secondary noxious,† per cent	Other, per cent			
Small Grains:								
Registered No. 1	Blue	99–100	None	0 01	0 10	0.10	1.00	90–100
Registered No. 2	Red	98–99	None	0 05	0.15	0.30	2.00	70–89
Alfalfa:								
Registered No. 1	Blue	99 3 100	None	0 01	0.10	0 20	0 70	90–100
Registered No. 2	Red	98.5–99 3	None	0 05	0 15	0.50	1 50	70–89

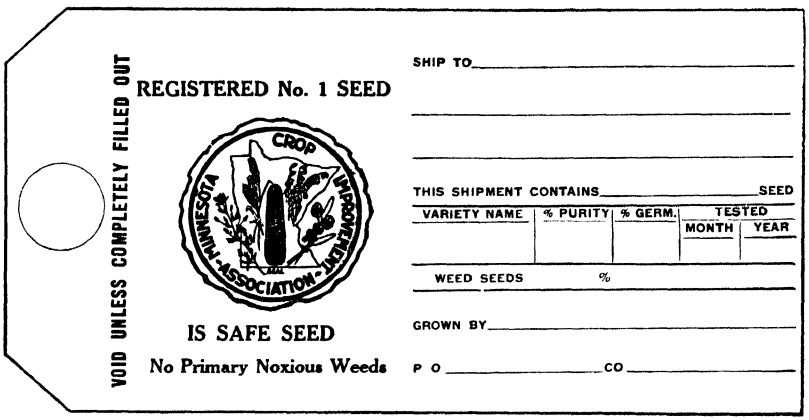
Grade	Tag	Germination	Grading	Moisture, per cent	Damage, per cent
Hybrid Corn:					
Registered No. 1	Blue	93–100	90–100	14	0.5
Registered No. 2	Red	75–92	75–89	15	1 5

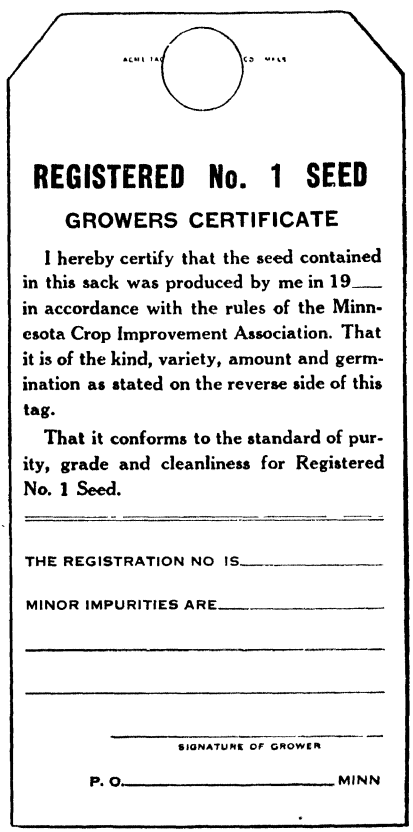
* Primary noxious weeds: Canada thistle, perennial sow thistle, quack grass, dodder, buckthorn, oxeye daisy, field bindweed, horse nettle, leafy spurge, Austrian field cress, false flax (in flax), and perennial pepper grass.

† Secondary noxious weeds: Wild mustard, French weed, wild oats, wild vetch, sheep sorrel, hedge bindweed, night-flowering catchfly, white cockle, and dragonhead mint.

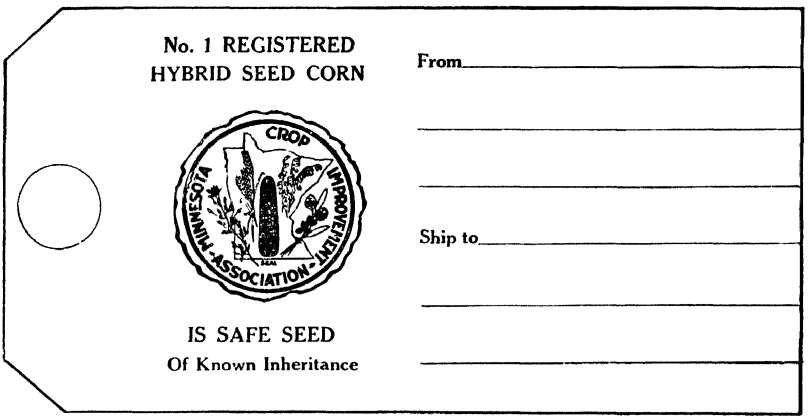
No primary noxious-weed seeds are allowed in either class of registered seed. The requirements for Registered No. 1 are considerably higher than for Registered No. 2.

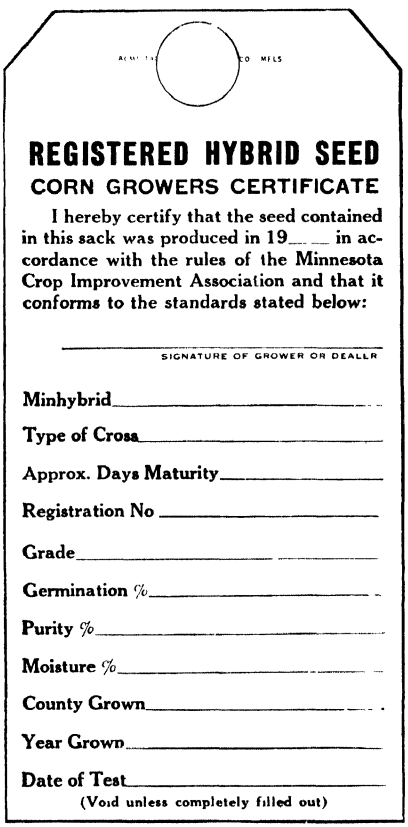
The blue tag given for Registered No. 1 may be illustrated as follows:





Tags used in registering hybrid corn seed are similar to those used for small grains. The material carried on the tag is different.





In addition to the blue- and red-tag grades of hybrid corn seed, the Minnesota Crop Improvement Association certifies hybrid corn seed for seed companies if they comply with requirements. A green tag is furnished bearing the same information as required for hybrids produced by growers of experiment-station hybrids. The tag carries the following statement:

> The seed in this container is certified on the affidavit of the producer filed with the Minnesota Crop Improvement Association that the parent lines used in the commercial crossing plot are of the same breeding and purity as those parental lines used in the hybrid tested in the official yield test. Regular inspections were made of the commercial crossing plot for isolation, detasseling and purity. Representative samples of the seed, as prepared for market, were inspected in the laboratory for moisture, germination, grading and physical appearance as required in the rules for certification.

Hybrids produced by seed companies that have been in official yield trials and that have given satisfactory performance are eligible for certification.

Seed (Tuber) Certification for Potatoes.—At the present time, there are 22 states actively engaged in the production of certified seed. In 10 states, certification is under the direction of the college of agriculture. These are Colorado, Idaho, Louisiana, Maryland, Michigan, Montana, New York, Oregon, Wisconsin, and Wyoming. In 9 states, the work is under the supervision of the state department of agriculture. These are California, Maine, Minnesota, New Jersey, North Dakota, Pennsylvania, Tennessee, Vermont, and Washington. In Nebraska, Utah, and South Dakota, growers' organizations have charge of certification; in Canada, the work is under the supervision of the Dominion Department of Agriculture, at Ottawa.

The objects of seed certification of potatoes include:

1. The production of high-grade seed potatoes that are relatively free from diseases and varietal mixtures and that are well graded.
2. Increased yield and better quality that follow the use of good seed stock, relatively free from disease.
3. More satisfactory prices of seed stock to careful growers of certified seed.
4. Better methods of production of tubers used for seed.

Sufficient fees are charged for inspection so that the cost of the work is paid for largely by the producer of certified seed.

The principal steps in potato-seed certification, as carried out in Maine, are briefly summarized.

Potatoes eligible for certification should be grown on land that was not in potatoes the previous year and on fields isolated by 250 ft. from other potatoes. It is recommended that certified seed be used to plant the field and that such seed be disinfected with corrosive sublimate. It is required that the crop be well cared for and be kept reasonably free from weeds and from injury by insects. It is required also that the field be sprayed with Bordeaux mixture to control late blight.

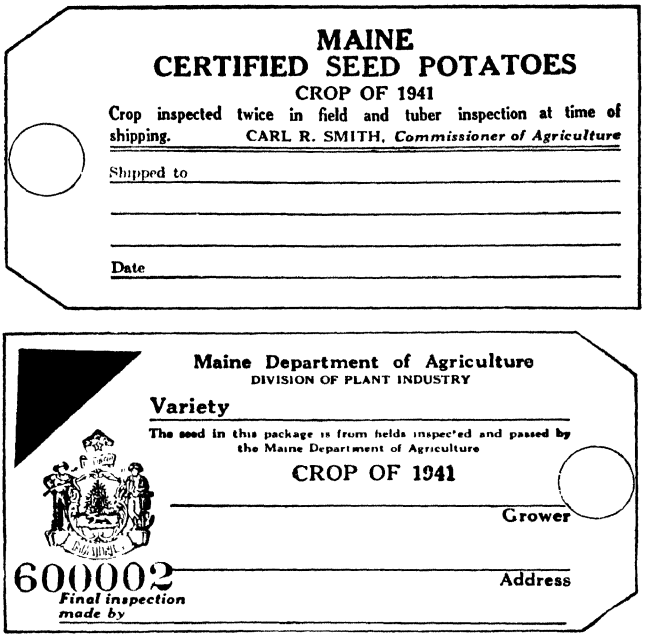
Two field inspections of the crop are made; the tolerances allowed for various diseases and varietal mixtures are given in the summary.

Tolerances allowed for diseases and varietal mixtures	First inspection, per cent	Second inspection, per cent
Leaf roll	2	1
Mosaic	3	2
Spindle tuber	2	2
Yellow dwarf	0 5	0.5
Total virus diseases	5	3
Blackleg	2	1
Wilt	2	1
Total of all diseases	6	4
Giant hills	...	1
Varietal mixtures	1	0.25

It is expected that the grower will remove diseased hills or varietal mixtures after each inspection.

At shipping time, a third inspection must be made. Maine-certified seed potatoes shall be equal or exceed U. S. Grade No. 1 to be eligible for certification.

The blue tag used to designate certified seed is illustrated here.



CHAPTER XVIII

SOME COMMONLY USED MEASURES OF TYPE AND VARIABILITY

Statistics are being used extensively in the reduction of data and interpretation of results from plant-breeding experiments. Whenever a large number of observations are obtained, it will be difficult to grasp the full importance of these observations because of their number. Consequently, the individual observations are replaced by a few statistics that convey all or most of the information available from the experiment in a form readily comprehended.

One of the commonest uses of statistics for the plant breeder is in their application to field trials where a considerable number of varieties are grown under comparable test. In such trials, it is necessary to determine the averages of yield and other characters and to estimate the significance of differences. As usually carried out, the first test is to determine whether there is a significant difference in the performance of any of the varieties. If the statistical method used indicates that all varieties have the same performance, within the limits of the accuracy of the study, no further comparisons are worth while. If there is a significant difference in performance, *i.e.*, if the odds are rather great that the difference in performance would not occur by chance alone, the next step is to compare individual varieties. For the plant breeder, this often will consist of a comparison between new selections that have been placed under test recently and a standard variety that has been shown previously to be the most desirable variety available.

Before the investigator can make these and other comparisons of a similar nature, it is necessary to learn the meaning of certain statistical terms and the method of their calculation.

DEFINITION OF STATISTICAL CONSTANTS

The commonest statistics are the mean and mode as measures of type and standard error, standard deviation, and variance as measures of variability.

The mean, or arithmetic average, is the sum of the measures or observations divided by their number.

The mode is the class of greatest frequency in a series.

The standard error is a measure of variability in terms of the units of measurement. The reliability of a particular statistic is determined by its standard error. The smaller the standard error in relation to the magnitude of the statistic the greater the confidence that may be placed in the significance of that statistic.

The standard deviation is similar to standard error except that it frequently refers to the infinite population rather than to any sample drawn from that population.

The variance is the square of the standard deviation or standard error.

The coefficient of variability is a measure of variability expressed in percentage of the mean, making it possible to compare the relative variability of two populations with widely different means.

CALCULATION OF MEAN, STANDARD ERROR, VARIANCE, AND COEFFICIENT OF VARIABILITY

The calculation of these statistics will be illustrated by using data given by Mercer and Hall (1911) on yield of 500 small plots of the same variety of wheat harvested from one field. In Table 32 is given the frequency of occurrence of plots where the yields have been grouped into classes of 0.2 lb. per plot.

In the calculations that will be illustrated, S means summation, f is the frequency or number of plots having a certain yield, x is the class-center value, and N is the total number of plots.

The formula for the calculation of the mean is as follows: Mean yield $= \bar{x} = S(fx)/N$.

In the problem: $S(fx) = S[(4 \times 2.8) + (15 \times 3.0) \cdots + (4 \times 5.2)] = 1974.6$. To obtain the mean yield, this value is divided by N, where N is the total number of plots. Numerically, this would be $1974.6 \div 500 = 3.9492$ lb. per plot.

The mode is the class with greatest frequency. In this problem, the modal class is 4.0 lb. Plus and minus deviations from the modal class often are similar in their frequencies; *i.e.*, the distribution is frequently symmetrical about the modal class. In pure-line material, these deviations are the result of the interaction of favorable and unfavorable environmental influences,

the number of individuals or plots where all conditions are favorable, or all unfavorable, being much smaller than those with part favorable and part unfavorable. In segregating lines, there may be also variation due to heritable causes, and in some cases a frequency distribution may show a bimodal curve.

TABLE 32.—FREQUENCY OF PLOTS WITH YIELDS GROUPED INTO 0.2-LB. INTERVALS

Class center of yield, x	Number of plots, f	fx	fx^2
2 8	4	11.2	31.36
3 0	15	45 0	135 00
3 2	20	64 0	204 80
3.4	47	159 8	543 32
3 6	63	226.8	816 48
3.8	78	296 4	1126 32
4 0	88	352 0	1408.00
4.2	69	289 8	1217.16
4 4	59	259 6	1142.24
4.6	34	156.4	719.44
4.8	11	52.8	253 44
5 0	8	40.0	200 00
5 2	4	20.8	108.16
Total.....	500	1974.6	7905.72

The common measures of variation are the standard error and variance, the latter being the square of the standard error.

The standard error is given by $s = \sqrt{\frac{S(fx^2) - S(fx)\bar{x}}{N - 1}}$, where S, f, x, and N have the same designations as above. From the foregoing table, this would be $\sqrt{\frac{7905.72 - (1974.6)(3.9492)}{499}} = 0.464$ lb. The foregoing formula also may be expressed as $\sqrt{\frac{S[f(x - \bar{x})^2]}{N - 1}}$. This is the standard error of a single determination. In practice, the value of the mean $\bar{x}$ used in the correction factor $S(fx)\bar{x}$ must be calculated with sufficient accuracy so that, when multiplied by the total, the product is accurate to the place desired. Usually it is more convenient to calculate the correction factor in the form $[S(fx)]^2/N$.

In Fig. 36 is given a histogram of the frequency of plots with different yields. In the same figure is superimposed the normal frequency distribution. This smooth curve is an estimate of the distribution of the infinite population from which these 500 plots are considered a sample.

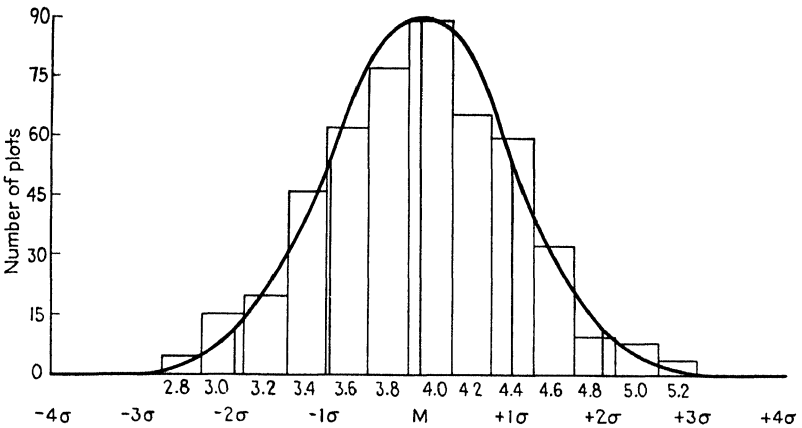


FIG. 36.—Yield in pounds per plot with ordinates drawn at ± 1, 2, 3, and 4 times the standard deviation.

In the illustration given, and by the use of tables such as Sheppard's (Pearson 1924), it is possible to determine the percentage of the area under the normal curve cut off by erecting perpendiculars from the base line to the curve at distances of 1, 2, or 3 standard deviations (1σ, 2σ, or 3σ, as illustrated in Fig. 36) from the mean, or M, of the curve, where σ is the standard deviation. From such tables, we find that the area between the two lines erected at plus and minus 1 times the standard deviation will be 68.27 per cent of the total area. It can be said, then, that the probability of an observation falling within $\pm 1\sigma$ will be given by $P = .6827$. The probability of an observation falling outside $\pm 1\sigma$ will be $1.0000 - .6827 = .3173$. In like manner, the probability of an observation falling within $\pm 2\sigma$ will be .9545, and within $\pm 3\sigma$ it rises to .9973. In the problem considered in Table 32, the mean was 3.95, and the standard error was 0.464. One can say, therefore, that the probability of an observation falling within the limits set up by the mean $\pm 2\sigma$, or $3.95 + 2(0.464) = 4.88$ and $3.95 - 2(0.464) = 3.02$, or between a yield of 3.02 and 4.88 lb. is 0.9545. The probability of

the yield of a plot, selected at random, falling outside the limits 4.88 and 3.02 would be .0455. Stated in another way, it may be said that the chances of an observation falling within these two limits will be .9545:.0455, or approximately 21:1. There is 1 chance in 22 of a plot, selected at random, falling outside the limits 4.88 and 3.02. The chances of an observation exceeding 4.88 would be 43:1. Stated in terms of probability, it would be .0455 ÷ 2 = .02275, or in a little over 2 per cent of the cases by chance alone would a plot selected at random yield over 4.88 lb.

The standard error of a mean is given by $s/\sqrt{N}$ where s = standard error of a single determination and N = the number of observations from which the mean is determined. It is obvious that the standard error of a mean must be smaller than the standard error of a single determination, since there will be less variation among means than among single observations.

The coefficient of variability (C.V.) is a relative measure of variability in percentage. C.V. = $(s \times 100)/\bar{x}$, where s and $\bar{x}$ are the standard error and mean for the sample. It is of value in comparing the variability of populations with different means or differing in units of measurement.

In many cases, the investigator will be interested in a comparison between the means of two varieties or treatments or in a comparison of a selection with the standard. It is essential to place these comparisons on the basis of probabilities. The usual procedure is to compare the difference with its error and determine the probability that a difference as great as or greater than that observed could be due to chance alone.

The standard error of a difference (s_{diff}) is given by the formula $\sqrt{s_a^2 + s_b^2 - 2r_{ab}s_a s_b}$, where a and b represent the two treatments being compared and r is the correlation between separate measurements of the quantities. When $r = 0$, the formula becomes $\sqrt{s_a^2 + s_b^2}$. When $s_a = s_b$ and $r = 0$, $s_{\text{diff.}}$ becomes $s\sqrt{2}$. In this and in other similar problems, the significance of a difference is determined by comparing it with the standard error of the difference. These problems will be taken up in detail in later chapters.

CORRELATION COEFFICIENT

The coefficient of correlation r is used as a measure of the degree of association between two characters worked with at the same

time. Perfect positive correlation is +1, and perfect negative correlation is −1; no correlation is given by $r = 0$; intermediate values denote association of an intermediate degree. A convenient working formula is

$$r_{xy} = \frac{S(xy) - S(x)S(y)/N}{\sqrt{S(x^2) - [S(x)]^2/N}\,\sqrt{S(y^2) - [S(y)]^2/N}}$$

where x represents the measures of one variable or character and y the other and all other letters have the same meaning as before. The calculaton of simple, partial, and multiple correlation coefficients will be given in a subsequent chapter.

COMPARISON OF DIFFERENCES BY THE *t* TEST

The t test provides the usual method for testing the significance of the difference between two means. Such problems are common in plant breeding. The statistic t is defined as a difference expressed in terms of the standard error of the difference. If we have two varieties, or treatments, from which the means are different, we shall wish to know in what proportion of the cases a difference as great as or greater than that observed can be expected to occur as a result of deviations due to random sampling.

Tests of this type fall into two classes: (1) when the samples are paired and (2) when the samples from one of the two varieties or treatments are not paired with those of the other. Both tests will be illustrated with data obtained in Minnesota from strip plantings of two varieties of wheat. Seed of the varieties Thatcher and Marquillo was sown in adjacent single strips of one drill width in each of many fields in the state. The purpose of the test was partly demonstrational and was partly to obtain comparative yields on many different farms. The yield was determined from comparable samples from each variety in each strip. In Table 33 are given the yields of these two varieties for 12 of the many farms on which tests were made and the sums and differences of the two varieties.

Since the two varieties were grown in paired plots, this fact will be utilized in the statistical analysis of the differences.

In this problem, differences in yield between Thatcher and Marquillo were determined for each of the 12 comparisons, and

the mean difference, called $\bar{x}$, divided by the standard error of this difference to obtain the value of t.

TABLE 33.—YIELDS OF THATCHER AND MARQUILLO WHEAT TESTED IN COUNTY DEMONSTRATION TRIALS IN MINNESOTA IN 1935

Farm number and county	Yield, bu. per acre		Sum	Difference
	Thatcher	Marquillo		
1. Roseau	24.4	17.5	41 9	6.9
2. Mahnomen	27.9	15.1	43 0	12.8
3. Traverse	28.2	21.6	49 8	6.6
4. Bigstone	19 8	18.2	38 0	1.6
5. Stevens	23.1	21.6	44 7	1.5
6. Stevens	22 9	13.7	36 6	9.2
7. Pope	25.6	24.8	50.4	.8
8. Kandiyohi	28.7	27.8	56.5	.9
9. Kandiyohi	26 2	25.2	51.4	1.0
10. Kandiyohi	25.7	19.2	44.9	6.5
11. Renville	37.0	34 0	71 0	3.0
12. Yellow Medicine	31.5	25 2	56 7	6 3
Sum	321.0	263 9	584.9	57.1

The calculated mean difference was obtained by dividing $S(x)$ by N, or 57.1 by 12, which gives a mean value of 4.76 bu. In other words, Thatcher yielded, as an average of the 12 trials, 4.76 bu. per acre more than Marquillo.

The standard error of a difference was obtained by the same formula as that presented previously, except that $f = 1$, where

$$s = \sqrt{\frac{S(x^2) - [S(x)]^2/N}{N - 1}}.$$

From Table 33, $S(x^2)$ was computed by squaring each of the 12 differences to give 437.85, and the value of s becomes $\sqrt{\frac{437.85 - [(57.1)^2/12]}{11}} = 3.89$. The standard error of the mean difference is $3.89/\sqrt{12}$ or 1.12.

The statistic t (the mean difference divided by its standard error) is given by $t = \frac{4.76}{1.12} = 4.25$.

In a determination of the significance of this difference, it is necessary to introduce the concept of degrees of freedom. That

term is used in the sense of independent comparisons. In calculating the standard error of a difference for the 12 comparisons, or differences in yield between Thatcher and Marquillo, only $N - 1$ deviations can vary, one being fixed by the sample mean. The degrees of freedom are 1 less than the number of comparisons, or 11 in this case.

Referring to Appendix Table I for 11 degrees of freedom, we find the values of t for the 5 and 1 per cent points to be 2.20 and 3.11, respectively. Values of t as great as these give odds of 19:1 and 99:1, respectively, against a difference as great as this occurring by chance alone. In this problem, with a value of $t = 4.25$, the chances are much greater than 99:1 that the difference is not due to chance.

In some cases, it may be desirable to compare the yields of two varieties when they are not grown in paired comparisons. Fisher (1938) has given a method where the value of t is calculated by comparing the mean difference in yield of the two varieties with its standard error, where $\bar{x}_1$ and $\bar{x}_2$ are the mean yields, s = the standard error, and N_1 and N_2 are the number of plots of each variety. Then

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s}\sqrt{\frac{N_1 N_2}{N_1 + N_2}}$$

where s^2

$$= \frac{1}{(N_1 - 1) + (N_2 - 1)}\{[S(x_1^2) - S(x_1)\bar{x}_1] + [S(x_2^2) - S(x_2)\bar{x}_2]\}.$$

For such comparisons of means, the number of plots of each variety need not be equal. Calculating the sums of squares of the 12 yields of Thatcher and Marquillo, we obtain 219.55 and 365.31, respectively. Then

$$s = \sqrt{\frac{219.55 + 365.31}{11 + 11}} = 5.16$$

$$t = \frac{4.76}{5.16}\sqrt{\frac{144}{24}} = \frac{4.76}{2.11} = 2.26$$

The degrees of freedom for comparing these two varieties in unpaired comparisons are 2 less than the sum of the number of trials of the two varieties, or 22 in this case. Entering the t table for $t = 2.26$ and 22 degrees of freedom, it is seen that the observed value of t lies between $P = .05$ and $P = .01$. The

chances that a difference as large as the observed would occur through random sampling would be less than 5 in 100 and more than 1 in 100.

It sometimes happens that in comparing two means based on the same number of observations, the data may be analyzed according to either method illustrated above. If either method gives a significant value of t, its testimony should not be ignored. With the paired relationship, the degrees of freedom will be one-half as large as when the data are not paired. This will result in a larger difference being required to reach the minimum level of significance because of the reduced number of degrees of freedom. If the correlation between paired plots is sufficiently high, the standard error of the difference will be reduced sufficiently so that the minimum level of significance is smaller in spite of the reduced degrees of freedom.

CHAPTER XIX

FIELD-PLOT TECHNIC

After making the initial selections from promising material, the final test in plant-breeding studies will consist of comparable trials in which the selected material will be compared with standard varieties. Although special technics may be adopted for studying such characters as winter hardiness and drought resistance, disease resistance, and other special qualities, it will be necessary in most cases to make comparable yield trials under actual field conditions. In these cases, the field becomes the experimental laboratory. The usual method consists of the use of small plots, adequately replicated and dealt with in such a manner as will give a reliable index of comparable yielding ability under actual farm conditions. In order to obtain the desired results, it has been found necessary to handle the experimental field, insofar as possible, in a manner that will approach the practices in use by the better farmers, *i.e.*, to follow established principles of farm management. Some of the important considerations may be summarized:

1. As far as possible, the soil and climatic conditions of the experimental field should be similar to those under which the crop will be grown by farmers.

2. A system of crop rotation should be followed that approaches, as closely as feasible, that used by the better farmers.

3. A bulk crop sown after the experimental trial aids in keeping the soil in a uniform state of fertility.

4. Competition between varieties and strains in the experimental trial must be eliminated or its effects controlled by randomization or by grouping varieties of like nature.

5. Satisfactory methods must be devised for handling the experimental plots, including weighing or counting the seed for planting, sowing, cultivating, harvesting, and threshing the crop.

6. Replication and the calculation of a standard error of the experiment aid in furnishing a basis for reliable conclusions, and

a well-designed experiment helps in controlling the effect of soil heterogeneity.

Some of these points seem self-evident; others need to be explained in greater detail. Each experiment must be planned on the basis of the information desired. All that will be attempted is the formulation of principles of wide application.

In order to determine the adaptation of varieties to actual farm conditions, it is necessary to make the field trials in various regions. For this purpose, it has become a standard practice to develop branch stations or test on selected farms in representative regions in order to test the new strains under those conditions to which they will be exposed after introduction to the farmer.

These experimental fields should be operated according to approved farm-management practices. Among these practices, the importance of an adequate system of conserving soil fertility will be generally appreciated. Crop rotation will be desirable in many cases.

CROP ROTATION FOR EXPERIMENTAL FIELDS

The system of rotation used should be similar to that recommended as a desirable farm practice. Several such rotations may be illustrated, although they are representative only of desirable practices for certain specific types of farming.

For the corn-yield trials at Minnesota, a 3-year rotation is practiced. In this case, one-third of the land is used in the yield trials. The rotation is as follows:

1. Corn-yield trials. Farm manure is applied, and superphosphate is added at the rate of 100 lb. per acre.
2. Small grain follows corn, this field being used to increase seed of a recommended variety.
3. Sweet clover is sown with the small grain and used for pasture or hay the third year.

Rotations have been developed for the final trials of spring and winter wheat made in $\frac{1}{40}$-acre plots that have proved satisfactory and that are similar to systems in use by Minnesota farmers. These need not be given in great detail.

Spring Wheat	Winter Wheat
1. Yield trial	1. Yield trial
2. Clover hay	2. Bulk corn for silage
3. Bulk corn for silage	3. Oats and peas for hay

Manure is added to the silage corn, which aids in maintaining soil fertility. Clover is planted with the spring wheat, and the first crop of clover is used for hay the following year, the second crop being turned under. The oats and peas for hay are harvested sufficiently early to permit adequate preparation of the land for the winter wheat. A part of this winter-wheat series is used for the winter-wheat breeding nursery.

At Cornell, the following general rotation for the experimental trial of cultivated crops has been used. The plan is as follows:

1. Soybeans as green manure, turned under.
2. Silage-corn-yield trials, manured, with addition of superphosphate, or yield trials of cabbage.
3. Yield trials of soybeans, field beans, sunflowers, or corn.

Such a rotation results in high fertility, and the yields are high if the seasonal conditions are satisfactory. This plan is followed because high yields are expected by New York farmers who grow silage corn and the other cultivated crops worked with.

In the rotations for these cultivated crops, as well as in the small grain trials at Cornell, variety trials may be made on the same fields for 2 consecutive years, and then a legume is turned under the third season. The small-grain trials are in a 3-year rotation, consisting of the following: (1) rod-row trials of oats, (2) rod-row trials of wheat, (3) clover cut off once and then plowed under.

These rotations will serve to illustrate methods that have proved fairly satisfactory. Although it is better to have a separate rotation for each yield trial, where the yield comparison of a crop occupies the position taken by that crop in a desirable crop sequence for the locality, it is not always possible to follow this plan because of insufficient cropland.

SOIL HETEROGENEITY

One of the difficulties encountered in field experiments is due to the fact that uniform soil conditions seldom exist, if ever, even over small portions of a field. Soil heterogeneity, as measured by yield of crops grown on a field and harvested as small plots, may be due to topography of the field, soil moisture, variation in fertility, or previous cropping practice.

In 1915, J. Arthur Harris proposed a criterion for measuring soil variation that he called a coefficient of soil heterogeneity.

Five years later Harris (1920) reported the results of tests made on published data involving a wide variety of crops and characters from experiments conducted over the entire world and demonstrated clearly that soil heterogeneity is practically universal. In concluding his paper, Harris stated, "The demonstration that the fields upon which the plot tests have been carried out in the past are practically without exception so heterogeneous as to influence profoundly the yields of the plots emphasizes the necessity for greater care in agronomic technic and more extensive use of the statistical method in the analysis of the data of plot trials if they are to be of value in the solution of agricultural problems." The many studies conducted since that time have amply substantiated these conclusions.

Uniformity trials, or blank tests, have been used extensively in studying the nature and extent of soil heterogeneity. In such uniformity trials, the field is planted to a single variety and harvested as small plots. The entire field is planted at the same rate of seeding, and cultural practice is the same over the entire area. The unit plots harvested can then be grouped to form plots of varying size and shape, the only variable being size or shape of the plots. Cochran (1937) published a catalogue of uniformity-trial data, listing 191 uniformity trials with field experiments, the data from 135 having been published.

The nature of soil heterogeneity may be demonstrated in graphical form by means of contour maps drawn from data obtained in uniformity trials. An example of such a contour map, drawn from data on yield of sugar beets in a uniformity trial, was given by Immer and Raleigh (1933) and is reproduced in Fig. 37.

In this study, the yields of six-row plots, each 2 rods long, were used. Points deviating by −15, −10, −5, 0, +5, and +10 per cent from the mean yield were interpolated between the centers of the plots and the contour map drawn by connecting these points. Figure 37 shows in a graphic way that fields that may appear to be very uniform are rather heterogeneous from the standpoint of productivity, as measured by yield on small areas. Such contour maps demonstrate graphically that soil variability is, to a certain extent, regular over small areas. There is a sort of "regular irregularity" to the fertility contours. The use of

different sizes or shapes of plots would change the contour map, but the general characteristics would remain the same.

The extent of soil heterogeneity may be measured by determining the degree of correlation of yields of near-by plots. Hayes and Garber (1927) presented data giving the correlation coeffi-

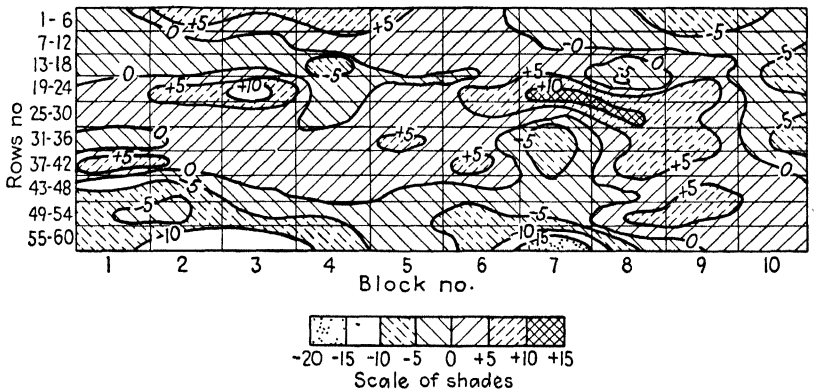


FIG. 37.—Contour map of weight of sugar-beet roots in a uniformity trial from 100 six-row plots each 2 rods long; contour lines drawn through the points deviating by −15, −10, −5, 0, +5, and +10 per cent from the mean weight.

cient between adjacent rod rows within tests with oats, spring wheat, and winter wheat and between rod rows separated by one or more plots. These data are reproduced in Table 34.

It is evident that the correlation is greatest between adjacent plots and decreases as the distance between the plots is increased. However, there was a sensible correlation between the yield of rod-row plots separated by as much as 10 rows.

Harris (1920), using the intraclass correlation coefficient, made an extensive study of soil heterogeneity, using data from uniformity trials obtained by numerous investigators. The extent to which contiguous plots resemble each other was measured in terms of intraclass correlation, the larger the coefficient the greater the degree of soil heterogeneity. The results are given in Table 35.

The data given in Table 35 are only a small part of those available but are presented to emphasize the usual extent of soil heterogeneity in plot studies.

The method of calculating simple correlation coefficients will be taken up in Chap. XX. The method of intraclass correlation will be illustrated here. Harris calculated the intraclass cor-

TABLE 34.—CORRELATION OF PERCENTAGE YIELDING ABILITY IN NEAR-BY PLOTS OF OATS, SPRING WHEAT, AND WINTER WHEAT, 1924

Crop	Correlation of	Correlation coefficient
Oat-rod rows	Adjacent plots	.572 ± .025
	Separated by 1	.490 ± 029
	Separated by 2	.407 ± .034
	Separated by 3	.412 ± .035
	Separated by 4	.264 ± .041
	Separated by 10	.275 ± .057
Spring-wheat rod rows	Adjacent plots	.618 ± .023
	Separated by 1	.518 ± .028
	Separated by 2	.454 ± .030
	Separated by 3	.383 ± .034
	Separated by 4	.449 ± .034
	Separated by 10	.429 ± .060
Winter-wheat rod rows	Adjacent rows	.552 ± .068
	Separated by 1	.293 ± .028
	Separated by 4	− .114 ± .118

TABLE 35.—CORRELATION COEFFICIENTS PRESENTED BY HARRIS THAT EXPRESS THE EXTENT OF SOIL HETEROGENEITY IN DIFFERENT LOCALITIES AND WITH DIFFERENT CROPS

Crop	Character	Size of plot	Investigator	Correlation coefficient
Wheat	Yield, grain	5.5 by 5.5 ft.	Montgomery, Nebr.	.603 ± .029
Wheat	Nitrogen content	5.5 by 5.5 ft.	Montgomery, Nebr.	.115 ± .044
Oats	Yield, grain	1/30 acre	Kiesselbach, Nebr.	.495 ± .035
Mangels	Yield, roots	1/200 acre	Mercer and Hall, England (Rothamsted)	.346 ± .042
Mangels	Yield, leaves	1/200 acre	Mercer and Hall, England (Rothamsted)	.466 ± .037
Potatoes	Yield	Rows, 72 ft., 7 in. long	Lyon	.311 ± .043
Corn	Yield, grain	1/10 acre	Smith, Ill (1895)	.830 ± .019
Alfalfa	Yield, hay			
	1913, first cutting	0.085 acre	Scofield, Huntley Experiment Farm, Montana	.407 ± .059
	1913, second cutting	0.085 acre		.343 ± .062
	1914, first cutting	0.085 acre		.602 ± .045
	1914, second cutting	0.085 acre		.657 ± .040

relation coefficient (which he designated a coefficient of soil heterogeneity) from the formula appropriate for analysis of a symmetrical correlation table. The manner of calculating the intraclass correlation as given by Fisher (1938) is illustrated from the simple illustration given below, assuming data from 16 plots in a field divided into four blocks of four each.

4	5	4	3
5	6	5	4
6	6	5	5
5	7	6	4

The sum of the 16 plot yields is 80 and the mean is $80 \div 16 = 5$. The total sum of squares is calculated from $S(x^2) - S(x)\bar{x}$, where x = individual plot yields, $\bar{x}$ = mean yield, and S = summation, or $416 - (80)(5) = 16$. The sums of the yields of the four blocks of four plots each are 20, 16, 24, and 20. Squaring these four sums, dividing by the number of plots in each sum, and subtracting the correction factor $S(x)\bar{x}$ gives the sum of squares between blocks. Numerically, this is $163\frac{2}{4} - 400 = 8$. The sum of squares within blocks is obtained by subtraction. The degrees of freedom for total and block variation will be 1 less than the number of total plots or blocks. An analysis of variance is given in Table 36.

TABLE 36.—ANALYSIS OF VARIANCE

Variation	Degrees of freedom	Sum of squares	Mean square	*F*
Between blocks	3	8	2.667	4.00
Within blocks	12	8	0 667	
Total	15	16		

The mean square is obtained by dividing the sums of squares by the respective degrees of freedom. *F* is the mean square for blocks divided by the mean square for variation within blocks.

If the mean square within blocks is set equal to B and the mean square between blocks equal to $(kA + B)$, where k is the number of plots per block, the intraclass correlation coefficient will be given by

$$\frac{A}{A + B}$$

In this illustration, $B = 0.667$, $k = 4$, and $kA + B = 2.667$. Therefore $kA = 2.000$, and $A = 0.500$. The intraclass correlation will be

$$\frac{0.500}{0.500 + 0.667} = +.428$$

The significance of the intraclass correlation coefficient may be determined from a comparison of mean squares between and within blocks. In this case, the mean square between blocks divided by the mean square within blocks gives a value for F of 4.00. The meaning of F will be discussed in greater detail in Chap. XX. An F of 4.00 is in excess of the 5 per cent point (Appendix Table II) for $n_1 = 3$, and $n_2 = 12$ degrees of freedom, n_1 and n_2 being the degrees of freedom for the larger and smaller mean squares, respectively. The intraclass correlation therefore may be judged significant. This coefficient presents in correlation form the ratio of the variance between and within blocks. It expresses the average correlation of plots within each group of adjacent plots that are studied.

It is important to know whether there is a tendency for plots that produce low yields in one season to produce low yields in succeeding seasons, etc. The results given by Harris and Scofield (1920, 1928) indicate a tendency for plots to yield in a similar manner from year to year, although there were some exceptions. Garber, McIllvaine, and Hoover (1926) found the interannual correlation between the yields of oat hay on 270 plots in 1923 and the yield of wheat grain on the same plots in 1924 was +.364. Garber and Hoover (1930) found that the interannual correlation between yields of plots in uniformity trials and yields of crops in a rotation test on the same field in succeeding years was positive in each test made. The differences in natural productivity of the plots persisted over a period of several years.

Summerby (1934) presented the most extensive data on a study of permanence of difference in yields of crops over a period of years. The interannual correlation coefficients were mostly positive, but 13 out of 143 were negative, and 3 of these exceeded the 5 per cent point. As a result of his study, Summerby concluded, "Under the conditions of this experiment the use of preliminary uniformity trials for the purpose of adjusting yields of subsequent experiments by regression is only rarely as effective in increasing precision as is the use of the same amount of land and labor in replicating the experiment in the year of the trial."

COMPETITION

Plants growing along the sides and ends of plots frequently are larger than those in the middle of the plot because of greater available fertility or water supply. This is true particularly when the plots are adjacent to uncultivated areas or are surrounded by alleys. The extent and nature of this border effect is important in comparative crop tests.

Arny and Hayes (1918) and Arny (1921, 1922) studied the extent of border effect in multiple-row plots. In Table 37 are presented data obtained by Arny and Hayes from plots seeded with a grain drill in rows 6 in. apart. Eighteen-in. alleys surrounded the plots with a roadway at each end. The plots were 17 drill rows wide and were trimmed to 132 ft. in length. Each of the outside border rows was harvested separately, and the yields compared with those of the 13 central rows. The plants on the end, to a depth of 1 ft., were cut off.

TABLE 37.—COMPARISON OF AVERAGE YIELD OF OATS, WHEAT, AND BARLEY HARVESTED FROM BORDER ROWS AND CENTRAL ROWS OF PLOTS 132 × 8.5 FT.

Source	Oats		Wheat		Barley	
	Number of plots	Yield per acre, bu.	Number of plots	Yield per acre, bu.	Number of plots	Yield per acre, bu.
Outside border rows....	44	132.0	20	55.0	16	97.7
Inside border rows.....	44	88.0	20	41.0	16	64 5
Central 13 rows.....	44	71.4	20	27.5	16	42.9

It is clear that border effect may profoundly influence yield. Not all varieties were affected alike by surrounding alleys. The rank order in yield frequently may not be the same with and without border rows removed. Arny (1922) found that sowing winter grains in the spring in the alleys between spring-grain plots reduced border effect somewhat but did not prevent it completely. Removing the outside two rows, 6 in. apart, before harvesting for yield removes the larger part of the border effect and gives a more accurate idea of the expected yields under good farm conditions.

In variety trials in small plots, usually no alley is left between plots. Varieties with different habit of growth consequently grow adjacent to one another. Hayes and Arny (1917) demonstrated that the border rows of tall varieties in three-row plots replicated three times that grew adjacent to short varieties yielded more than the central row. Often the intervarietal competition was sufficient to cause differences of 4 or 5 bu. per acre in yield of the border rows as compared with the central row of the same variety.

Kiesselbach (1918) gave some illuminating information of the effect of intervarietal competition in small grains. He compared the differences in yield of adjacent single-row plots of different varieties with the differences in yield of the same varieties grown in alternate blocks, each consisting of three to five rows. The yield of border rows was in some instances included in the yield of the blocks. His results are summarized in Table 38.

The data illustrate clearly that competition between adjacent varieties in one-row plots may seriously disturb the yields of these varieties. In general, the varieties grown in alternate rows show greater differences than when grown in alternate blocks of multiple-row plots. The higher yielding varieties usually benefit by planting in adjacent single rows, but this is not always the case.

Intervarietal competition can be overcome by planting multiple-row plots and discarding the border rows before harvesting. Three- to five-row plots with one border discarded on each side are commonly used. At Minnesota, the three-row plot has become the standard for rod-row trials. Grouping the material so that only strains of similar habit of growth and maturity are grown adjacent to one another will tend to reduce

the effect of competition and permit harvest of the entire plot. Such procedure implies, however, that the experimenter can determine in advance whether competition will or will not be a disturbing factor, an assumption for which there is no evidence, usually, prior to the time the experiment is conducted.

TABLE 38.—SUMMARY OF RELATIVE GRAIN YIELDS OF VARIETIES TESTED IN SINGLE-ROW PLOTS AND ALSO IN BLOCKS CONTAINING SEVERAL ROWS

Varieties compared in alternating rows and in alternating blocks	Year of test	Ratio of variety 1 to variety 2		
		Alternating rows	Alternating blocks	Competing in same hill
Turkey Red (1) and Big Frame (2) winter wheat	1913	100:107	100:97	
Turkey Red (1) and Big Frame (2) winter wheat	1914	100:85	100:97	
Turkey Red (1) and Nebraska No. 28 (2) winter wheat	1913	100:107	100:107	
Turkey Red (1) and Nebraska No. 28 (2) winter wheat	1914	100:63	100:85	
Kherson (1) and Burt (2) oats	1913	100:130	100:112	
Kherson (1) and Burt (2) oats	1914	100:139	100:101	
Kherson (1) and Swedish Select (2) oats	1913	100:82	100:77	
Kherson (1) and Swedish Select (2) (2) oats	1914	100:89	100:93	
Hogue's (1) and Pride of the North (2) corn	1912	100:66	100:85	100:47
Hogue's (1) and Pride of the North (2) corn	1914	100:38	100:53	100:26
Hogue's (1) and University No. 3 (2) corn	1914	100:90	100:98	100:99
Crossbred Hogue's (1) and inbred Hogue's (2) corn	1916	100:31	100:37	100:21

Tysdal and Kiesselbach (1939) found that plots of alfalfa, of the varieties Hardistan and Ladak, drilled in rows 7 in. apart, were definitely subject to serious interplot varietal competition. This could be overcome by removal of border rows. They found little or no differential interplot competition in rows 12 in. apart.

Immer (1934) reported the results of tests with two varieties of sugar beets, Old Type and Extreme Pioneer. These two

varieties were grown in alternate single-row plots and alternate four-row plots, with the central two rows harvested. The rows were 22 in. apart in 1930 and 1931 and 20 in. apart in 1932. As an average of 10 replications in each of 3 years, Old Type yielded 3.78 ± 0.44 tons more than Extreme Pioneer in single-row plots but only 1.78 ± 0.31 tons more in four-row plots. The difference between these two differences was 2.00 ± 0.54 tons. The higher yielding variety profited at the expense of the lower in single-row plots as compared with a multiple-row plot from which the border rows were discarded.

The effect of competition within and between hills of corn is frequently very striking. Kiesselbach (1923) has furnished some pertinent information on the relative yields of one- , two- , and three-plant hills adjacent to or surrounded by hills of variable stand. These data are given in Table 39.

TABLE 39.—RELATIVE YIELDS OF ONE-, TWO-, AND THREE-PLANT HILLS ADJACENT TO OR SURROUNDED BY HILLS OF VARIABLE STAND

Type of comparison	Total number of hills averaged	Number of ears per 100 plants	Relative yield, per cent
3-plant hills surrounded by 3-plant hills. . .	598	89	100
2-plant hills surrounded by 3-plant hills. . .	120	99	82
1-plant hills surrounded by 3-plant hills . .	80	141	61
3-plant hills adjacent to 1 hill with 2 plants	360	91	102
3-plant hills adjacent to 1 hill with 1 plant.	302	94	107
3-plant hills adjacent to 1 blank hilll	366	94	114

The four hills adjacent to a blank hill increased by 4 × 14 = 56 per cent. Therefore, only 44 per cent of the potential yield of the missing hill was lost. This recovery is without consideration of the slight increase that would be expected from hills on the corners of the blank hill. A one-stalk hill surrounded by three-stalk hills yielded but 61 per cent as much as three-stalk hills surrounded by three-stalk hills, resulting in a loss in yield of 39 per cent. The four three-plant hills adjacent to a one-plant hill increased in yield by 4 × 7 = 28 per cent of normal and resulted in a net loss of 39 − 28 = 11 per cent because of the inclusion of a one-stalk hill and the four adjacent three-stalk

hills in the yield determination. In the case of two-stalk hills, the loss in yield was 18 per cent, but 8 per cent of this was recovered in the four adjacent three-stalk hills.

Brewbaker and Immer, in Minnesota (1931), studied the effect of missing hills, or hills with reduced stands, in inbred lines of corn and F_1 crosses. The data for an average of 2 years for the F_1 crosses are given in Table 40. The average yield of the check hills was 76.3 bu. per acre in 1928 and 78.4 bu. in 1929. The yield of three-stalk hills surrounded on the four sides as well as four corners was used as a check for the other yield comparisons.

TABLE 40.—EFFECT OF COMPETITION WITHIN AND BETWEEN HILLS OF F_1 CROSSES OF CORN

Type of comparison	Number of hills	Yield in percentage of check
3-plant hills between 2 blank hills	117	108 9
3-plant hills opposite 1 blank hill	78	105 6
3-plant hills blank hills on 2 corners	69	100 2
3-plant hills between two 1-stalk hills	94	104 5
3-plant hills opposite one 1-stalk hill	72	103 7
3-plant hills between two 2-stalk hills	76	102 5
3-plant hills opposite one 2-stalk hill	77	101.5
2-plant hills surrounded by 3-plant hills	87	75 4
1-plant hills surrounded by 3-plant hills	96	41 0

Each of four three-stalk hills adjacent to a blank hill increased in yield by 5.6 per cent. The increase in yield of each of the four adjacent three-stalk hills due to one- or two-stalk hills on one side was 3.7 and 1.5 per cent, respectively. One-stalk hills and two-stalk hills yielded but 41.0 and 75.4 per cent, respectively, as much as three-stalk hills. It follows, therefore, that the inclusion of one- or two-stalk hills introduces a greater error than to ignore their effect on the yield of the surrounding three-plant hills. Harvesting only hills with a perfect stand and surrounded by perfect-stand hills sometimes reduces the number of hills available for harvest materially, resulting in an increase in the intraplot error. A part of the variation (frequently the major portion) in stand between F_1 crosses is due to random causes and will tend to equalize over an average of the several

replications. In view of these considerations, a working rule has been adopted at Minnesota of harvesting only three-plant hills surrounded by one- , two- , or three-plant hills in yield tests with F_1 crosses. A further reason for this procedure is that frequently one wishes to obtain the potential yield of the crosses without the influence of differential stand resulting from differences dependent upon the ability to produce a stand as influenced by different inbred lines.

In yield tests with three-way or double crosses that are to be grown commercially, a frequent practice is to determine the percentage stand of each hybrid in the yield tests, but the yields are based on the actual production of the plots without correction for stand.

Kiesselbach and Weihing (1933) studied the effect on yield of variable number of plants per hill in plots all having the same total number of plants, under Nebraska conditions, to determine the effect of variable stand on yield. The corn was planted with an average of three plants per hill in the plots. The treatments consisted of uniform three-plant hills and variable stands of 2-4, 1-3-5, and 1-2-3-4-5 plants per hill. The average yields of these four treatments, for a 14-year period, was 49.9, 50.6, 49.3, and 50.0 bu. per acre. It appears from these data that the yield per plot is essentially the same provided that the total stand is the same.

SIZE AND SHAPE OF PLOTS

In general, there are two kinds of experimental plots. Nursery plots usually are small and are planted by hand or with special nursery equipment and are cared for by hand cultivation. Field plots generally are larger and are adapted to the use of standard farm machinery. The distinction between these two types of plots may, in some instances, be more or less arbitrary.

For cereal grains, the rod-row plot is relatively standard in this country. Such plots usually are 18 ft. long, and the plants to a distance of 1 ft. at both ends of the plots are removed before harvest. In the case of wheat, the yield in grams of a single row 16 ft. long with 1 ft. between rows multiplied by 0.1 converts the yield to bushels per acre. Rod-row plots vary, in different types of experiments and in different stations, from single to five-row plots. In the case of multiple-row plots, one border row from

each side of the plot frequently is discarded to correct for possible differential intervarietal competition. Rod-row plots frequently are sown at the same rate of seeding recommended for commercial farm planting in that area, although a lighter rate of seeding is practiced by some workers. The distance between rows usually is greater than that used by commercial farm drills, since hand cultivation between the rows is needed to control weeds satisfactorily.

Field plots vary from about $\frac{1}{100}$ to $\frac{1}{10}$ acre in size. They offer somewhat greater opportunity than single or three-row plots to observe crop behavior under conditions comparable with those found on farms. In general, experience has shown that rod-row tests and large field plots compare very favorably in testing for varietal differences, provided that adequate precautions are taken to guard against competition and other errors.

Increasing plot size will, in general, decrease the error of a single-plot yield. On the other hand, increasing plot size will increase the land area in the blocks and, consequently, soil heterogeneity in the blocks. The variability among sets of plots of varying size will depend on the balance between these two opposing tendencies.

Studies of the variability among plots varying in size and shape are numerous. It is found generally that increasing replication will decrease the standard error more rapidly than increasing size of plots. Plots relatively small, adapted to the type of nursery equipment available, should be used. The size used will vary with the crop and conditions of the test. Field trials under irrigation, for example, frequently require some modification in procedure not necessary in tests without irrigation.

In general, it appears that long and narrow plots lead to a lower error than square ones. Christidis (1931), from theoretical considerations, suggested that long, narrow plots would control soil heterogeneity better than plots more nearly square, occupying the same area of land. He examined data from six uniformity trials and found support for this hypothesis. Others have found essentially the same thing. The relative efficiency of plots of varying shape will depend on the direction of the fertility contour lines across the field. If the predominant direction of these contours is known, long narrow plots planted at right angles to the direction of the fertility contours would lead to the low-

est error, since variability within blocks is then reduced to a minimum.

In tests involving varieties of naturally cross-pollinated crops, the size of plot needed to obtain a given precision will depend somewhat on the nature of the material. Bryan (1933) found that about one-half as many plants or hills of corn were needed to obtain the same level of precision in tests of the yielding ability of crosses between inbred lines as compared with open-pollinated varieties. Since the plants from crosses between inbred lines are less variable than plants from open-pollinated varieties, the variability within plots of hybrids will be reduced, and this, in turn, will decrease the calculated variability between plots.

REPLICATION

Replication serves two purposes. (1) Replication increases the precision of the experiment, since the mean of several replications provides a more accurate measure of varietal performance than does a single plot. (2) From replicated trials, it is possible to calculate an estimate of error of the experiment.

The number of replications used will depend on the variability of the soil, the variability of the material to be tested, the degree of precision desired, and the amount of seed available.

In experiments in which randomized blocks are used, the standard error of the mean will be $s/\sqrt{N}$, where s is the standard error of a single determination and N is the number of plots of each variety or treatment. The standard error of the mean may be reduced, therefore, to whatever level is desired through sufficient replication. After the standard error of a single determination for plots of the desired size and shape is learned, the number of replications needed to reduce the standard error to a certain degree of accuracy may be obtained by the formula

$$\left(\frac{\text{Standard error of a single determination}}{\text{Standard error desired}}\right)^2$$

METHODS OF MAKING YIELD TRIALS USED IN MINNESOTA

If careful notes are taken on all characters such as lodging, winter hardiness, and reaction to diseases and if varieties or strains that are inferior to the standard variety in any important

respect are freely discarded before making extensive yield trials, this, in many cases, will reduce the number of strains that must be studied for yielding ability.

Where only a limited number of varieties or strains are included, randomized blocks have considerable advantage over other methods. The number of varieties included in each block should be sufficiently small so that all varieties in each block are under similar conditions. At Minnesota, 25 or fewer varieties in each block have given relatively satisfactory results. One or two standard varieties are grown in each block where more than a single group of 25 varieties are under trial, and the standard error is computed separately for each group of randomized blocks. The methods of making yield trials may be illustrated as they are carried on at Minnesota for spring wheat.

First Year.—Rod-row trials are made at University Farm, St. Paul, in single-row plots with two to four replications, depending upon the amount of seed available. In these studies, check plots of standard varieties are grown in each randomized block and in each of the three disease nurseries: (1) for studies of reaction to stem and leaf rust, (2) for studies of reaction to bunt, root rot, and black chaff, and (3) for studies of reaction to scab. For the scab trials, the rows are grown under a tent, which increases humidity and makes infection relatively heavy. Notes are taken on agronomic characters in the yield trials, and all varieties or strains inferior to the check in any important respect are eliminated.

Second Year.—Rod-row trials are made at University Farm in randomized blocks with not more than 25 varieties per block and the inclusion of two or three standard varieties in each block. Three-row plots replicated four times are used, the central row of each plot being harvested for the yield trial and the border rows for milling and baking tests. The data are analyzed by means of the analysis of variance to determine if there are significant differences in yield. A calculated standard error is obtained by dividing the standard error of a single determination by the square root of the number of replicates, *i.e.*, randomized blocks. Significant differences are considered to be twice the standard error of a mean difference. In these and in subsequent trials, all varieties are grown also in the disease nurseries and are eliminated when found inferior to the standard varieties in any

important respect. The more promising varieties are tested also in milling and baking trials.

If the number of strains available for testing is not too great and there is sufficient seed, they may be tested the first or second year at several stations.

Third to Fifth Year.—Three-row plots are used as in the second year, and trials are made in randomized blocks with three replications at each of four stations, University Farm, St. Paul, and the branch stations at Morris, Crookston, and Waseca, making 12 replications in all. The data taken are similar to that outlined for the second year at University Farm. A mixture of seed from the four stations is used for the milling and baking tests.

Sixth to Eighth Year.—The more promising varieties in the rod-row trials are increased and placed in $\frac{1}{40}$-acre plot trials in randomized blocks, with three replications at each station, tests being conducted at the same four stations and, in addition, at Grand Rapids and Duluth. Very promising varieties in the rod-row trials may be advanced to the $\frac{1}{40}$-acre plot trials before the sixth year. Fewer than 20 varieties are in these yield tests, as a rule, even though several thousand plant selections are grown yearly. This means that varieties are discarded freely.

The standard errors for each trial are averaged for a series of years and stations, considering that each trial may be a test of the desirability of the new variety for use in some region in Minnesota. The formula for the standard error of an average that is used is $1/N \sqrt{s_1^2 + s_2^2 \cdots s_n^2}$, where s_1^2, s_2^2, etc., are errors for each season and station and N is the number of trials made. This generalized average error is used as a standard error for the average yield of the varieties at several stations and for several years.

Varieties grown in similar trials are then compared on the basis of a standard error of a difference. The error is calculated by multiplying the generalized average error by $\sqrt{2}$. The difference in yield between any two varieties is obtained and the significance of this difference determined by the t test, as explained previously.

CHAPTER XX

RANDOMIZED BLOCKS, LATIN SQUARES, AND χ^2 TESTS

Experimental designs and statistical methods that are in most common use by the plant breeder will be illustrated for the beginning student who has not had extensive mathematical training or previous experience in the application of biometrical methods to plant-breeding problems.

TESTS IN RANDOMIZED BLOCKS

One of the simplest experimental designs for testing the yielding capacity of a group of varieties, particularly if the number is not unduly large, say less than 25, is that of randomized blocks In such designs, the varieties are grown in random order in each of several complete replication series or blocks, the number of replications used depending on the degree of precision desired for the comparisons of the variety means.

R. A. Fisher stated that randomization of the order of varieties in a block must be followed if an unbiased estimate of error is to be obtained. Tedin (1931) tested the validity of this assumption and confirmed Fisher's conclusion. Before describing studies in randomized blocks, a method of obtaining a randomized order for planting will be given where 20 varieties are being compared.

The following plan is one suggested by Fisher (1937) in "The Design of Experiments." Use a pack of cards numbered from 1 to 100, and arrange them in random order by repeated shuffling. If 20 varieties are to be tested, they are then numbered from 1 to 20 and cards drawn from the pack. Divide the number of the card drawn by 20, and the remainder will give the variety to be planted in the first plot. Suppose that the first card drawn is 33. Dividing by 20 leaves 13 as a remainder, and variety 13 is taken first. Suppose that the second card is 40, giving zero as a remainder. Numbers divisible by 20 correspond to variety 20. Cards are drawn in this manner until the order of all varieties has been obtained. The remainder corresponding to any

variety is disregarded after its first occurrence in the block. After one block arrangement has been completed, the cards are reshuffled before drawings are made for the second block.

Since 100 is divisible by 20, each variety will be represented in the pack five times. If 19 varieties were to be placed in randomized order, the same pack of cards could be used but cards with numbers above 95 discarded, since 95 is directly divisible by 19, leaving no remainder.

Tables of random numbers, when available, such as those given by Tippett (1927) and Fisher and Yates (1938) can be used instead of a pack of cards in order to save labor. In such tables, one may start at any point and proceed in any direction, taking each pair of digits to represent the numbers of a card in a pack of 100. In these tables, 00 will be used in place of 100.

The analysis of variance is used to determine the significance of results obtained in randomized-block designs. By this procedure, developed by Fisher (1938), the total variation is separated into a number of components attributable to known or controlled sources of variation and leaving a residual portion due to uncontrolled causes and called the error.

Data from a randomized-block trial with 10 varieties of barley, reported by Immer, Hayes, and Powers (1934), will be used to illustrate the computation. The data are given in Table 41.

TABLE 41.—YIELDS IN BUSHELS PER ACRE OF 10 VARIETIES OF BARLEY GROWN AT UNIVERSITY FARM, ST. PAUL

Variety	Block number			Sum	Average
	I	II	III		
Manchuria	29 2	25 0	26 8	81 0	27.0
Glabron	44 6	39 1	45 5	129.2	43.1
Svansota	33 9	39 4	32.1	105 4	35.1
Velvet	36 7	41 0	42 0	119 7	39 9
Trebi	41 2	31 9	36 6	109 7	36 6
Minn. 457	45.8	38.8	45 2	129.8	43.3
Minn. 462	35 8	36.0	38 0	109 8	36 6
Peatland	38 5	29 6	30 2	98 3	32.8
Minn. 475	15.5	32.8	25 7	74.0	24 7
Barbless	44.3	37.4	36.2	117.9	39.3
Sum	365.5	351.0	358 3	1074.8	

First it will be necessary to calculate the sum of squares of deviations for the total variation and for blocks and varieties. The sum of squares for error is obtained by subtracting the sum of squares for blocks and varieties from the total.

The total sum of squares will be given by $S(x^2) - S(x)\bar{x}$. Squaring the 30 individual-plot yields and summing gives $S(x^2) = 39{,}949.06$. The total, or $S(x)$, $= 1074.8$. The mean $\bar{x} = 1074.8 \div 30 = 35.826{,}667$. Then, the correction term $S(x)\bar{x}$ will be $1074.8 \times 35.826{,}667 = 38{,}506.50$. Care must be used to calculate the mean to sufficient accuracy. This difficulty is overcome by squaring the total and dividing by the total number of plots. Thus, $\frac{[S(x)]^2}{N} = \frac{(1074.8)^2}{30} = 38{,}506.50$. The total sum of squares will be $S(x^2) - S(x)\bar{x} = 39{,}949.06 - 38{,}506.50 = 1442.56$.

The sum of squares for blocks is obtained by adding the squares of the block totals, dividing by the number of plots in each total, and subtracting the correction term. Expressed as a formula, this will be $\frac{S(x_b^2)}{10} - S(x)\bar{x}$, where x_b refers to the block totals. Numerically, this is $\frac{385{,}170.14}{10} - 38{,}506.50 = 10.51$.

The sum of squares for varieties is calculated in a manner similar to that for blocks. If x_v represents the variety totals, the sum of squares will be $\frac{S(x_v^2)}{3} - S(x)\bar{x}$. This is $\frac{118{,}668.36}{3} - 38{,}506.50 = 1049.62$.

In these and other calculations where totals are used, it is necessary to calculate the sums of squares on a unit basis. This is accomplished by dividing the squares of the totals by the number of unit plots in each before subtracting the correction term.

The complete analysis of variance is given in Table 42.

The degrees of freedom for blocks, varieties, and total are 1 less than the number of blocks, varieties, and total plots, respectively. The degrees of freedom for error will be the remainder after subtraction of the degrees of freedom for blocks and varieties from the total. In randomized-block trials, the error degrees of freedom will be the product, also, of the degrees of freedom for blocks multiplied by the degrees of freedom for varieties.

The column of mean squares in Table 42 is obtained by dividing the sums of squares by the appropriate degree of freedom. These values are the estimated variances expressed on a single-plot basis. The standard error of a single plot is the square root of the error mean square, *i.e.*, $\sqrt{21.25} = 4.61$.

TABLE 42.—ANALYSIS OF VARIANCE OF YIELDS OF 10 VARIETIES OF BARLEY IN A RANDOMIZED BLOCK TRIAL

Variation due to	Degrees of freedom	Sum of squares	Mean square	F	s
Blocks	2	10.51	5.26	0 25	
Varieties	9	1049.62	116.62	5.49*	
Error	18	382 43	21 25	.....	4.61
Total	29	1442 56			

* Exceeds the 1 per cent point.

To determine whether block or varietal differences are significant, the variance ratios, or values of F, are calculated. These are the mean squares for blocks and varieties each divided by the error mean square. For comparing variety and error mean squares, use is made of the table of F published by Snedecor (1940) and reproduced as Appendix Table II. The expected value of F for n_1 = degrees of freedom for the larger mean square and n_2 = degrees of freedom for the smaller mean square, or in this case for $n_1 = 9$ and $n_2 = 18$, is found to be 2.46 and 3.60 at the 5 and 1 per cent points, respectively. The observed value of F, for comparing variety and error mean square, exceeded the 1 per cent point. Therefore, we may conclude that less than once in 100 trials could varietal differences as great as those observed be obtained by chance. We may say, therefore, that some of the varietal differences are highly significant, using the term highly significant when the observed value of F exceeds the 1 per cent point.

The mean square due to blocks was less than that for error. This shows that the variation between block totals was less than expected through random sampling alone. Whenever the mean square for blocks or varieties is less than error mean square, there is no point in calculating and testing the significance of F. The removal of variability due to blocks removes differences due to

the placing of one block on land of different productivity from another. This makes it possible to place each block on a separate field if one so desires.

Standard malting barley varieties in Minnesota are Manchuria, Velvet, and Barbless. The question may be asked, what are the chances that these three varieties were significantly different in yield in this test? The mean yields of Manchuria, Velvet, and Barbless were 27.0, 39.9, and 39.3 bu. per acre, respectively. The standard error of a mean of three plots is obtained by dividing the standard error of a single determination (s) by $\sqrt{N}$, where N is the number of replications. The standard error of the difference between two means of three plots is obtained by multiplying the standard error of the mean by $\sqrt{2}$. This is on the basis that the error in bushels, as calculated, will be the same for all varieties regardless of their mean yield. The standard error of the difference between two means may be calculated from $\sqrt{\frac{2s^2}{N}} = \sqrt{\frac{2 \times 21.25}{3}} = 3.76$ bu., where N is the number of plots in each mean. To determine the minimum difference required for odds of 19.1 or 99:1, we must multiply the standard error of the difference by the value of t for the degrees of freedom for error in the analysis of variance at the 5 and 1 per cent points, respectively. It is seen in Appendix Table I that for 18 degrees of freedom $t = 2.10$ at the 5 per cent point and 2.88 at the 1 per cent point. Multiplying 3.76 by 2.10 and 2.88 gives 7.90 and 10.83, respectively. Yields of Velvet and Barbless were 39.9 and 39.3 bu. respectively, whereas Manchuria yielded only 27.0 bu. Since Velvet and Barbless differed in yield from Manchuria by 12.9 and 12.3 bu., respectively, we may state that the chances are less than 1 in 100 that these differences in yield are due to chance.

If, instead of comparing the differences between these varieties, irrespective of the direction of the difference, we want to determine the probability that Velvet and Barbless exceeded Manchuria in yield, we should need to use one tail only of the probability curve. In that case, we should use t for $P = .10$ and .02 instead of .05 and .01 to obtain the probability of a deviation in one direction only occurring in 5 and 1 per cent of the time through random sampling.

The plant breeder usually compares the performance of new strains with standard varieties whose performance has been well established. If the odds were 19.1, for example, that the difference in yield between Barbless and Manchuria, plus or minus, are not due to chance, the odds would be 39:1 [(2 × 19 + 1):1] that Barbless exceeded Manchuria. The odds that the increased yield of Barbless over Manchuria is not due to chance would be two times, plus one, that found from the *t* table.

Some practices essential to satisfactory randomized-block tests can be summarized at this point. Size of plot used may be decided on two general bases. Sufficient plants to give a measure of the characters of the strain or variety and a plot of convenient size from the standpoint of handling and cost are the bases for selection of a plot of proper size. In general, the number of varieties in a block should not be much greater than 25, or the area of the block will be so great that soil heterogeneity may lead to too large a standard error. In simple plant-breeding experiments, when large numbers of strains are available for testing, the trials may be made by using randomized blocks with not more than 25 varieties in a block and the growing of one or more standard varieties in each block. New strains are compared with the standard by this method before comparing those that survive this test with each other.

LATIN SQUARES

The Latin square has been shown to be a desirable method of making precise comparisons when the number of treatments or varieties to be compared is small, *i.e.*, from about 4 to 10. Although of less general value for the plant breeder than randomized blocks, it is a desirable method for special experiments.

In a Latin-square design, there are as many replications as treatments. The treatments are arranged in a random order in a square or rectangle, with the restriction that each treatment can occur but once in each row and each column. For randomizing the order of the varieties, use may be made of a pack of cards, as described for randomized blocks, or by reference to the published sets of Latin squares [from 4 × 4 to 9 × 9, given by Fisher and Yates (1938)]. A Latin-square arrangement for five treatments, *A*, *B*, *C*, *D*, *E*, is illustrated below:

E	*B*	*D*	*C*	*A*
A	*C*	*E*	*B*	*D*
B	*E*	*A*	*D*	*C*
C	*D*	*B*	*A*	*E*
D	*A*	*C*	*E*	*B*

The degrees of freedom for an analysis of variance would be keyed out as follows:

Variation Due to	Degrees of Freedom
Rows	4
Columns	4
Treatments	4
Error	12
Total	24

The calculation of the sums of squares proceeds as outlined for randomized blocks. If x = the yield of each plot, x_r, x_c, and x_t the total yield of each row, column, or treatment, and p = the number of rows, columns, and treatments, the sum of squares for rows, columns, and treatments and the sum of squares for the total may be calculated as follows:

Sum of squares for rows	$\frac{S(x_r^2)}{p} - S(x)\bar{x}$
Sum of squares for columns	$\frac{S(x_c^2)}{p} - S(x)\bar{x}$
Sum of squares for treatments	$\frac{S(x_t^2)}{p} - S(x)\bar{x}$
Sum of squares for total	$S(x^2) - S(x)\bar{x}$

The sum of squares for error is obtained by subtracting the sum of squares for rows, columns, and treatments from the total.

The shape of plots in a Latin square need not be square. They may be rectangular in shape. If very long and narrow, however, the variation in soil fertility in the narrow direction will be small, and little will be gained by removing the variation in this direction. With plots for which the ratio of length to width is not extreme, removing variability in two directions usually will result in reduction in the error as compared with randomized blocks.

ESTIMATING THE YIELD OF A MISSING PLOT

It sometimes happens that the yield of some plot, or plots, is lost or is known to be unreliable. When that happens, it may be desirable to interpolate yields for the missing plots before the analysis of variance can be completed.

Yates (1933) has given a formula for estimating the yield of a missing plot. The interpolated yield is so calculated that the error variance is made a minimum. The formula appropriate for randomized-block trials is as follows:

$$X = \frac{pP + qQ - T}{(p - 1)(q - 1)}$$

where X = yield of missing plot.
p = number of treatments.
q = number of blocks.
P = sum of known yields of treatment with a missing plot.
Q = sum of known yields of block with a missing plot.
T = total yield of known plots.

As an example, assume that the yield of Minn. 462 in block II of Table 41 were missing. Then

$$p = 10$$
$$q = 3$$
$$P = 73.8$$
$$Q = 315.0$$
$$T = 1038.8$$

$$X = \frac{(10 \times 73.8) + (3 \times 315.0) - 1038.8}{(10 - 1)(3 - 1)} = 35.8$$

which is the estimated yield of the missing plot.

If two or more plots are missing, a method of approximation may be used based on the foregoing formula. With, say, two plots missing, a yield is assumed for one of the missing plots and the formula used to estimate the second. The assumed yield of the first missing plot is then erased and the formula used to estimate that. The yield estimated first is then recalculated and the same procedure applied in rotation to obtain the accuracy desired.

After obtaining the estimated yield of the missing plot, the analysis of variance is carried through in the usual manner except that 1 degree of freedom is subtracted from the error and 1 from

the total for each missing plot interpolated. In plant-breeding trials in randomized blocks, the data for the variety in which one or more plots are missing may be disregarded in computing the analysis of variance, providing that the degrees of freedom used are for the varieties actually used in the analysis.

In Latin squares, the formula for estimating the yield of a missing plot is

$$X = \frac{p(P_r + P_c + P_t) - 2T}{(p - 1)(p - 2)}$$

where X = missing plot yield.
p = number of rows, columns, or treatments.
P_r, P_c, P_t = sum of the known yields of the row, column, or treatment with a missing plot.
T = total yield of known plots.

SPLIT-PLOT EXPERIMENTS

In designing experiments involving two or more factors, the split-plot arrangement is often useful. The arrangement of the plots and the manner of calculation of the data will be illustrated, with the use of data obtained by A. C. Arny.

This experiment was one designed to determine the effect of varying the width between rows and spacing of seed within rows on the yield of soybeans. Four-row plots 132 ft. long were planted in rows 16, 20, 24, 28, 32, or 40 in. between rows. These long plots were then divided into four subplots of 33 ft. each and the soybeans spaced ½, 1, 2, or 3 in. apart within the subplots. The entire experiment was replicated four times, and only the central two rows of each four row plots were harvested. The field arrangement of the plots in block III is given below:

16	½	1	3	2
32	1	3	2	½
28	3	½	1	2
40	2	1	½	3
24	1	3	2	½
20	3	1	½	2

The order of the plot widths within each block was random, and the four spacings were randomized within each main or width plot. The dimensions of each block were 53⅓ by 132 ft.

In Table 43 are given the yields in bushels per acre for each plot, arranged in a convenient form for computation.

TABLE 43.—YIELD OF SOYBEANS IN BUSHELS PER ACRE

Block	Width of rows, in.	Spacing within rows, in.					Block total
		½	1	2	3	Sum	
I	16″	25 1	21.3	22.3	22.1	90 8	
	20″	21.8	22.7	22.2	22.8	89.5	
	24″	21.9	21.8	21 2	20.6	85.5	
	28″	21.2	20.4	20.4	17.9	79.9	
	32″	20.7	20.0	18.3	20.0	79 0	
	40″	19.5	18.3	17.5	16.3	71 6	496.3
II	16″	25.2	19.9	22.1	22 7	89 9	
	20″	21.9	21 3	22.1	22 9	88.2	
	24″	19 7	19 8	20 1	19.8	79 4	
	28″	20 8	21 2	18.8	20.6	81 4	
	32″	18.5	20.7	17 5	16.4	73 1	
	40″	18.5	18.2	19 8	15 9	72.4	484.4
III	16″	15 7	21.6	22.9	20.3	80 5	
	20″	22 0	20 4	22 4	20.7	85.5	
	24″	25 5	20.7	20 7	20 5	87.4	
	28″	21.5	19.9	20 5	20.9	82.8	
	32″	22.0	19.3	18.1	17.8	77.2	
	40″	20.5	16.4	17.5	18 5	72.9	486.3
IV	16″	23.8	29.0	12.3	23 5	88 6	
	20″	27.0	21.2	20.5	20.7	89 4	
	24″	23.5	20 0	22.3	19.8	85.6	
	28″	22 5	21.5	22 7	18 9	85.6	
	32″	23.9	18.4	20 7	18.7	81.7	
	40″	19 9	17 8	16.9	18.5	73.1	504 0
Sum.....		522.6	491.8	479 8	476.8	1971 0	1971.0

Squaring the 96 individual plot yields gives $S(x^2) = 41{,}045.92$. The correction term will be $S(x)\bar{x} = (1971.0)^2/96 = 40{,}467.09$. The total sum of squares is then $41{,}045.92 - 40{,}467.09 = 578.83$.

The block sum of squares will be

$$\frac{S(x_b^2)}{24} - S(x)\bar{x} = \frac{971{,}460.74}{24} - 40{,}467.09 = 10.44$$

where x_b^2 represents the squares of the block totals.

The sum of squares for the main or width plots is calculated from the column of sums on the right-hand side of Table 43. Thus,

$$\frac{90.8^2 + 89.5^2 + \cdots + 73.1^2}{4} - S(x)\bar{x}$$

$$= \frac{162{,}768.54}{4} - 40{,}467.09 = 225.05$$

The data in Table 43 are next assembled in the form given in Table 44 in order to obtain the sums of the four replications for each width of row and spacing within the rows.

TABLE 44.—TOTAL YIELD FOR EACH WIDTH OF ROW AND SPACING FOR THE FOUR REPLICATIONS

Width of rows, in.	Spacing within rows, in.				Sum	Average
	½	1	2	3		
16	89 8	91.8	79.6	88.6	349.8	21 9
20	92.7	85.6	87 2	87 1	352 6	22.0
24	90.6	82 3	84 3	80 7	337 9	21 1
28	86.0	83 0	82.4	78 3	329 7	20 6
32	85 1	78 4	74 6	72 9	311 0	19 4
40	78 4	70.7	71 7	69 2	290 0	18 1
Sum	522 6	491 8	479 8	476 8	1971 0	
Average...	21.8	20.5	20.0	19 9		

The sum of squares for widths of row will be $\frac{S(x_w^2)}{16} - S(x)\bar{x} = \frac{650{,}386.30}{16} - 40{,}467.09 = 182.05$, where x_w^2 is the square of the totals of 16 plots for each width.

The sum of squares for spacings will be $\frac{S(x_s^2)}{24} - S(x)\bar{x} = \frac{972{,}524.28}{24} - 40{,}467.09 = 54.75$, where x_s^2 is the square of the totals of 24 plots for each spacing.

Next, the 24 sums within Table 44 are squared and added. Thus $\frac{89.8^2 + 92.7^2 + \cdots + 69.2^2}{4} - 40{,}467.09 = 267.23$ for these 23 degrees of freedom. From this sum of squares is subtracted the sums of squares for width and spacing to give 267.23 − 182.05 − 54.75 = 30.43 as the sum of squares for the interaction of width × spacing.

The entire analysis of variance is given in Table 45.

TABLE 45.—ANALYSIS OF VARIANCE OF YIELD IN BUSHELS PER ACRE IN SPACING TRIAL WITH SOYBEANS

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Blocks	3	10 44	3.48	1.60
Width of row	5	182 05	36 41	16 78†
Error a	15	32.56	2 17 $= s_a^2$	
Main plots	23	225.05		
Spacing	3	54.75	18 25	3.67*
Width × spacing	15	30.43	2.03	
Error b	54	268 60	4 97 $= s_b^2$	
Total	95	578.83		

* Exceeds the 5 per cent point.
† Exceeds the 1 per cent point.

The degrees of freedom and sum of squares for error a are obtained by subtracting the degrees of freedom and sum of squares for blocks and widths from the main plots, which have 23 degrees of freedom and the sum of squares for which was 225.05. The degrees of freedom for error b will be 95 − 15 − 3 − 23 = 54. The sum of squares for error b is obtained by subtraction in like manner.

It is noted that the value of F for a comparison of the mean squares for widths of row with that of error a was highly significant. The mean square for spacings is compared with that of error b and exceeded the 5 per cent point but did not reach the 1 per cent point. The mean square for interaction of width × spacing was numerically less than the mean square for error b and clearly not significant.

From these data one may conclude that the differences in yield of soybeans in this test, planted in rows of different width, was independent of the spacing within the rows. The planting arrangement that would be expected to result in the highest yield would be the combination of highest average width per row and spacing within the row.

The standard error of the difference between two means for different widths of row would be

$$\sqrt{\frac{2 \times s_a^2}{16}} = \sqrt{\frac{2 \times 2.17}{16}} = 0.521$$

s_a^2 being divided by 16, since the means are based on that number of plots and multiplied by 2, since the standard error of a difference is desired. Since t at the 5 per cent point for 15 degrees of freedom is 2.13, the minimum level of significance would be $2.13 \times 0.521 = 1.11$ bu. The average yield of all plots with 20-in. rows was 22.0 bu., and the average yield for 28-in. rows was 19.4 bu. The difference of 1.4 bu. exceeded the minimum level of significance and may be judged significant.

The standard error of the difference between the means of two spacings would be

$$\sqrt{\frac{2 \times s_b^2}{24}} = \sqrt{\frac{2 \times 4.97}{24}} = 0.644$$

Since t at the 5 per cent point and 54 degrees of freedom is 2.00, mean differences in excess of $2.00 \times 0.644 = 1.30$ may be judged significant. Spacing of the soybeans 2 in. apart in the row resulted in a reduction of 1.8 bu. per acre over ½-in. spacing, a significant decrease.

Split-plot experiments are very useful when two or more factors are to be tested in one experiment and planting difficulties make it necessary that large plots be used for one factor, the large, or main, plots being split up for the second factor. Variety tests combined with dates of planting tests would be of this type, the dates of planting being the main plots and the varieties the subplots. Tests of fungicidal dusts on different varieties would often require rather large plots for the different dust treatments in order to control "drift" of the dust in application. These could well be the main plots and several varieties planted in subplots

within each main or dust treatment plot. For such tests, the split-plot design would be well suited.

CHI-SQUARE (χ^2) TESTS

Tests of Goodness of Fit.—The χ^2 test is a useful method for testing goodness of fit of Mendelian ratios, as pointed out by Harris (1912). The methods will be illustrated by various examples.

In the F_2 generation of a cross between a two-rowed variety of barley producing green seedlings (*VV LgLg*) with a six-rowed variety with light-green seedlings (*vv lglg*) the following number of plants was obtained in the four phenotypic classes:

V Lg	*V lg*	*v Lg*	*v lg*	Total
281	59	60	58	458

The general formula for calculating χ^2 may be written as

$$\chi^2 = \frac{S(O - C)^2}{C}$$

where S refers to summation, O is the observed frequency, and C is the expected or calculated frequency.

The test for deviations of the single factor ratio *Vv* may be made as follows:

Phenotype	Observed (O)	Calculated (C)	$O - C$	$\frac{(O - C)^2}{C}$
V	340	343.5	−3.5	0.036
v	118	114 5	3 5	0.107
Total	458	458.0	0 0	$\chi^2 = 0.143$

On entering the table of χ^2 (Appendix Table III) for 1 degree of freedom, it is found that the observed χ^2 lies between $P = 0.95$ and 0.50. The degrees of freedom are 1 less than the number of classes. A χ^2 value as great as the observed would be expected to occur between 50 and 95 times in 100 trials through errors of random sampling.

χ^2 for a 3:1 ratio may be calculated also from

$$\chi^2 = \frac{(A - 3a)^2}{3N}$$

where A = observed number in the dominant class.
a = number in the recessive class.
N = total number.

For the foregoing problem $\chi^2 = \frac{[340 - 3(118)]^2}{3 \times 458} = 0.143$, the same as found by the longer method.

Below are listed some formulas that may be useful for two class segregations.

Segregation Expected $A:a$	χ^2 Formula
1:1	$\chi^2 = (A - a)^2/N$
2:1	$\chi^2 = (A - 2a)^2/2N$
3:1	$\chi^2 = (A - 3a)^2/3N$
15:1	$\chi^2 = (A - 15a)^2/15N$
9:7	$\chi^2 = (7A - 9a)^2/63N$

The agreement between the ratio observed and the ratio expected on the basis of independent inheritance of the two factor pairs may be tested by calculating χ^2 for goodness of fit to a 9:3:3:1 ratio. The calculations are given in Table 46, with the use of the data from the experiment mentioned previously.

TABLE 46.—CALCULATION OF GOODNESS OF FIT TO A 9:3:3:1 RATIO

Phenotype	Observed (O)	Calculated (C)	$O - C$	$\frac{(O - C)^2}{C}$
V Lg	a = 281	257.625	23.375	2 121
V lg	b = 59	85.875	−26.875	8.411
v Lg	c = 60	85.875	−25 875	7 796
v lg	d = 58	28 625	29 375	30 145
Total	N = 458	458.000	0 000	χ^2 = 48 473

The calculated frequency for the four phenotypic groups will be 9/16, 3/16, 3/16, and 1/16 of the total, respectively. On entering the table of χ^2 (Appendix Table III) for 3 degrees of freedom, it is noted that the observed value of χ^2 = 48.473 greatly exceeds the 1 per cent point. In goodness-of-fit tests, such as the fore-

going, the degrees of freedom are 1 less than the number of classes. It may be concluded, therefore, that the deviation from a 9:3:3:1 ratio was highly significant.

A somewhat shorter method may be used for testing goodness of fit to a 9:3:3:1 ratio. Thus

$$\chi^2 = \frac{16(a^2 + 3b^2 + 3c^2 + 9d^2)}{9N} - N$$

where a, b, c, and d = observed frequencies as given in Table 46. Ther

$$\chi^2 = \frac{16[281^2 + 3(59^2) + 3(60^2) + 9(58^2)]}{9 \times 458} - 458 = 48.473$$

as before.

The nature of the deviation of the observed ratio from that expected on the basis of independent inheritance may be determined by separating χ^2 into its components. The 3 degrees of freedom for the goodness-of-fit test may be apportioned to: one for deviat ons of the *Vv* segregation from a 3:1 ratio, one for deviations of the *Lg lg* segregation from a 3:1 ratio, and one for detecting association (linkage) of the two factor pairs. Convenient formulas are

For *Vv* segregation	$\chi^2 = (a + b - 3c - 3d)^2/3N$
For *Lg lg* segregation	$\chi^2 = (a - 3b + c - 3d)^2/3N$
For linkage	$\chi^2 = (a - 3b - 3c + 9d)^2/9N$

The formula for deviations of single-factor ratios will reduce to the form given before. Substituting the observed ratios in these three formulas gives

For *Vv* segregation	$\chi^2 = 0.143$ for 1 degree of freedom
For *Lg lg* segregation	$\chi^2 = 0.073$ for 1 degree of freedom
For linkage	$\chi^2 = 48.257$ for 1 degree of freedom
For goodness of fit	$\chi^2 = 48.473$ for 3 degrees of freedom

In referring to the table of χ^2 (Appendix Table III), it is noted that the agreement of the two single-factor ratios with a 3:1 ratio is good. The χ^2 for linkage exceeds the 1 per cent point.

With the use of the product method for calculating linkage (Fisher 1938) and tables provided by Immer (1930), the per-

centage of recombination between these two factor pairs was 30.2 ± 2.7.

χ^2 for Independence.—The χ^2 test may be used to determine whether two characters, classified into two or more groups, are independent. The calculations will be illustrated with data obtained by Hayes, Moore, and Stakman (1939) in a study of inheritance of characters in crosses between varieties of oats.

Among the characters studied were plumpness of grain and type of awn. One of the parents, Bond, produced short, weak awns; the other parent, designated Double Cross, produced long, heavy awns. The two parents differed in plumpness of grain, as may be noted in the following table. Plumpness of grain is a visual note taken on a scale of 0 to 100 and has been found to be correlated significantly with yield.

TABLE 47.—PERCENTAGE OF PLUMPNESS OF KERNEL OF INDIVIDUAL PLANTS OF THE PARENT VARIETIES

Variety	Number of plants in plumpness classes				
	0–25	26–50	51–75	76–100	Total
Bond	1	6	54	61	122
Double Cross	5	28	26	..	59

In Table 48 are given the number of F_2 plants in different classes of plumpness and awn development.

TABLE 48.—FREQUENCY IN CLASSES FOR AWN DEVELOPMENT AND PERCENT OF PLUMPNESS OF GRAIN OF F_2 PLANTS IN THE CROSS OF BOND × DOUBLE CROSS

Plumpness, per cent	Awn classes		Total
	Weak	Intermediate	
0–50	46	8	54
51–75	165	44	209
76–100	120	27	147
Total	331	79	410
Proportion	0.80732	.19268	

To determine whether these two characters are independent, we may compare the observed frequencies with theoretical fre-

quencies calculated on the assumption of independence. The theoretical frequencies for the individual cells of the table are calculated so that they are in the same proportion to one another as they are in the totals of the rows and columns.

The theoretical frequency in the upper left-hand cell of the table will be the product of the two marginal totals divided by the grand total or $(331 \times 54)/410 = 43.60$. The other theoretical frequencies are calculated in a similar manner. To expedite the computations, the proportion of the grand total in each column may be calculated first. This is designated as "proportion" in Table 48. The proportion of weak and intermediate awn plants may then be multiplied by the margin totals for plumpness classes, *i.e.*, 54, 209, and 147, to obtain the theoretical frequencies. In Table 49 is given the computation of χ^2 for independence.

TABLE 49.—CALCULATION OF χ^2 FOR INDEPENDENCE OF AWN TYPE AND PLUMPNESS OF KERNELS

Observed frequency	Calculated frequency	$O - C$	$\frac{(O - C)^2}{C}$
46	43.60	2.40	.132
165	168 73	−3 73	.082
120	118 68	1 32	.015
8	10.40	−2.40	.554
44	40 27	3 73	.345
27	28.32	−1.32	.062
Sum 410	410.00	0.00	$\chi^2 = 1$ 190

Since χ^2 was calculated from fixed marginal totals, the degrees of freedom are $(r - 1)(c - 1) = 2$, where r and c refer to the number of rows and columns, respectively, in Table 48. On entering the table of χ^2 for 2 degrees of freedom, it is found that the observed χ^2 gives a value of P between .50 and .70. These data indicate, therefore, that there was no association between plumpness of kernel and development of awn in this segregating population. The χ^2 test is frequently useful in testing for independence of different characters in plant-breeding studies, when the data for each character are grouped in classes and entered as illustrated in Table 48.

CHAPTER XXI

CORRELATION AND REGRESSION IN RELATION TO PLANT BREEDING

SIMPLE CORRELATION

When data on two or more characters of a group of varieties or treatments are available, it frequently will be of value to determine the degree of association between them. This may be done by calculating the correlation coefficient. The coefficient of correlation can vary from $+1$ to -1, being zero when there is no association and increasing to $+1$ or -1 for complete association.

A method of computation will be illustrated by using data obtained in Minnesota from a study of the relationship between number of kernels per spikelet and yield of grain in bushels per acre in rod-row trials with spring wheat. Each plot consisted of three rows, and the central row only was used for obtaining the number of kernels per spikelet and yield. The number of kernels per spikelet was obtained from 100 spikes, selected at random, from each plot. The yield was computed from the central row of each three-row plot. The experiment was a randomized-block trial. The data are given in Table 50.

Before making a study of the extent of correlation between yield and number of kernels per spikelet, it will be of value to determine whether these strains of wheat differed significantly in the two characters being studied. This is accomplished through an analysis of variance. The results are given in Table 51.

The mean squares for varieties compared with error exceeded the 1 per cent point, indicating that highly significant differences in yield and number of kernels per spikelet existed among these 21 varieties. For neither character were there significant differences between blocks.

To calculate the correlation coefficient, it is necessary to determine the covariance. This is computed from the sums of products in a manner analogous to the computation of sums of squares. The sums of products are obtained from the sum of the products

TABLE 50.—NUMBER OF KERNELS PER SPIKELET AND YIELD IN BUSHELS PER ACRE, IN EACH OF THREE REPLICATIONS, IN ROD-ROW TRIALS OF SPRING WHEAT

Varieties or crosses	Kernels per spikelet					Yield, bu.				
	1	2	3	Total	Mean	1	2	3	Total	Mean
Marquis	1.5	1.5	1.4	4.4	1.47	22.7	22.3	28.8	73.8	24.6
Ceres	1 9	1.6	1.7	5.2	1.73	32.9	31.1	27.4	91.4	30.5
Hope	1.1	1 2	1.1	3.4	1.13	27.1	21.3	18.1	66.5	22.2
Ceres × Hope No. 1	1.4	1.7	1 4	4.5	1.50	19.4	19.2	23.7	62.3	20.8
Ceres × Hope No. 2	1.5	1.4	1.4	4.3	1.43	26.4	35.1	28.1	89.6	29.9
Ceres × Hope No. 3	1.5	1.4	1.3	4.2	1.40	26.2	36.4	29.3	91.9	30.6
Ceres × Hope No. 4	1.4	1.5	1.4	4.3	1.43	24.2	24.1	20.0	68.3	22.8
Ceres × Hope No. 5	1.4	1.4	1.3	4.1	1.37	23.8	26.3	24.3	74.4	24.8
Double Cross No. 80	1.3	1.2	1.4	3.9	1.30	26.8	25.7	30.5	83.0	27.7
Double Cross No. 85	1.5	1.3	1.4	4.2	1.40	22.6	26.6	19.9	69.1	23.0
Double Cross No. 86	1.4	1.5	1.3	4.2	1.40	23.2	23.5	28.8	75.5	25.2
Double Cross No. 97	1.4	1.4	1.4	4.2	1.40	32.4	27.5	28.1	88.0	29.3
Double Cross No. 98	1.4	1.3	1.5	4.2	1.40	26.1	25.3	30.7	82.1	27.4
Double Cross No. 99	1.4	1.5	1.3	4.2	1.40	22.1	28.1	28.6	78.8	26.3
Double Cross No. 100	1.5	1.5	1.3	4.3	1.43	27.1	28.3	26.8	82.2	27.4
Double Cross No. 102	1.4	1.3	1.4	4.1	1.37	27.1	30.8	28.9	86.8	28.9
Double Cross No. 103	1.4	1.5	1.4	4.3	1.43	26.9	29.1	22.6	78.6	26.2
Marquis × H44, No. 25	1.2	1.3	1.2	3.7	1.23	15.9	18.7	19.8	54.4	18.1
Marquis × H44, No. 33	1 1	1.2	1 3	3.6	1.20	27.9	27.9	23.5	79.3	26.4
Marquis × H44, No. 35	1.2	1.4	1.3	3.9	1.30	27.0	21.4	25.0	73.4	24.5
Marquis × H44, No. 40	1.3	1.2	1.2	3.7	1 23	22.6	23.3	24.0	69.9	23.3
Total	29.2	29.3	28.4	86.9	....	530.4	552.0	536.9	1619.3	

of the deviations of x and y from their means, which may be expressed as $S(x - \bar{x})(y - \bar{y})$. This is most conveniently calculated from $S(xy) - S(x)S(y)/N$, and the covariance is found by dividing the sum of products by the appropriate degrees of freedom. The sums of products may be either positive or negative.

An analysis of covariance is made in the same manner as an analysis of variance. Letting y and x represent yield in bushels per acre and number of kernels per spikelet, respectively, the total sum of products is given by $S(xy) - S(x)S(y)/N$. Multiplying each plot yield by the number of kernels per spikelet for that plot and summing gives 2242.16. Since $S(x) = 86.9$ and $S(y) = 1619.3$, the correction term will be obtained by multiplying 86.9 by 1619.3 and dividing by 63, which gives 2233.606. The total sum of products will be 2242.16 − 2233.606 = 8.554.

Letting x_b and y_b be the block totals for x and y, the sum of products for blocks will be $\frac{S(x_b y_b)}{21} - S(x)S(y)/N$, or 2233.773 − 2233.606 = 0.167.

If x_v and y_v are the variety totals for x and y, the sum of products for varieties will be $\frac{S(x_v y_v)}{3} - S(x)S(y)/N$, or 2243.937 − 2233.606 = 10.331.

TABLE 51.—ANALYSIS OF VARIANCE OF YIELD IN BUSHELS PER ACRE AND NUMBER OF KERNELS PER SPIKELET OF 21 VARIETIES OF SPRING WHEAT IN A RANDOMIZED-BLOCK TRIAL

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Yield per acre (y)				
Blocks	2	11 69	5.845	
Varieties	20	654.29	32.714	3.46*
Error	40	378 04	9.451	
Total	62	1,044.02		
Kernels per spikelet (x)				
Blocks	2	0 023	0.0115	1.40
Varieties	20	0.930	0.0465	5.67*
Error	40	0 330	0 0082	
Total	62	1.283		

* Exceeds the 1 per cent point.

The error sum of products is obtained by subtracting sums of products for blocks and varieties from the total.

In the following table are assembled the sums of squares (from Table 51) and sums of products for the different components of the total variation together with the correlation coefficients.

The coefficient of correlation will be given by

$$r = \frac{\text{sum of products of } xy}{\sqrt{\text{sum of squares of } y}\ \sqrt{\text{sum of squares of } x}}$$

The correlation between varieties is found to be

$$r = \frac{10.331}{\sqrt{654.29}\ \sqrt{0.930}} = +.419$$

The significance of a correlation coefficient may be determined by reference to Appendix Table V, where the degrees of freedom will be 2 less than the number of pairs. The variety correlation +.419 was slightly less than the value of $r = .433$ at the 5 per cent point for 19 degrees of freedom.

TABLE 52.—SUMS OF SQUARES AND PRODUCTS FOR YIELD IN BUSHELS PER ACRE AND KERNELS PER SPIKELET AND THE CORRELATION COEFFICIENTS

Variation due to	Degrees of freedom	Sums of squares or products			r
		y^2	xy	x^2	
Blocks	2	11.69	0.167	0.023	
Varieties	20	654.29	10.331	0.930	+.419
Error	40	378 04	−1.944	0.330	−.174
Total	62	1,044.02	8.554	1.283	

If it is desired to calculate a correlation coefficient for, say, yield and kernels per spikelet of varieties without performing an analysis of variance and covariance, a convenient formula is

$$r = \frac{S(xy) - S(x)S(y)/N}{\sqrt{S(x^2) - [S(x)]^2/N}\,\sqrt{S(y^2) - [S(y)]^2/N}}$$

The calculations, with the use of the mean kernels per spikelet and yield in bushels for the varieties in Table 50, will be illustrated. Multiplying the mean kernels per spikelet by the mean yield for each variety and summing gives $S(xy) = 747.773$. Summing the squares of the means for kernels per spikelet and yield in bushels gives $S(x^2) = 40.2199$ and $S(y^2) = 14{,}098.53$. Since the total of the means are $S(x) = 28.95$ and $S(y) = 539.9$, the correlation coefficient will be

$$r = \frac{747.773 - (28.95)(539.9)/21}{\sqrt{40.2199 - (28.95)^2/21}\,\sqrt{14{,}098.53 - (539.9)^2/21}} = +.423$$

This agrees closely with $r = +.419$ obtained in Table 52, the discrepancy being due to rounding off figures in recording the means.

If this formula for the correlation coefficient used above is multiplied by N/N

$$r = \frac{NS(xy) - S(x)S(y)}{\sqrt{NS(x^2) - [S(x)]^2}\sqrt{NS(y^2) - [S(y)]^2}}$$

which probably is the best form for rapid machine computation.

Numerically, this will be

$$r = \frac{21(747.773) - (28.95)(539.9)}{\sqrt{21(40.2199) - (28.95)^2}\sqrt{21(14{,}098.53) - (539.9)^2}} = +.423$$

LINEAR REGRESSION

The relationship between two variables may be expressed also by means of the regression coefficient. The regression coefficient gives the rate of change in one variable (the dependent variable) per unit rate of change in another (the independent variable). The regression coefficient is given by

$$b_{yx} = \frac{S(x - \bar{x})(y - \bar{y})}{S(x - \bar{x})^2} = \frac{S(xy) - S(x)S(y)/N}{S(x^2) - [S(x)]^2/N}$$

This may be expressed also as

$$b_{yx} = \frac{\text{sum of products of } xy}{\text{sum of squares of } x}$$

where b_{yx} = regression of y on x.

Numerically, the regression of yield on number of kernels per spikelet for the 21 varieties in Table 52 is $10.331 \div 0.930 = +11.109$. This means that as the number of kernels per spikelet of the varieties increased by 1.0, the yield of the varieties, on the average, increased by 11.1 bushels, or with an increase of spikelet number of 0.1 yield in bushels increased by 1.11.

The significance of a regression coefficient may be tested by means of an analysis of variance. The total sum of squares for varieties is given in Table 52. The test of significance of regression is given in Table 53. The sum of squares due to regression for varieties will be

$$\frac{(\text{Sum of products of } xy)^2}{\text{Sum of squares of } x} = \frac{(10.331)^2}{0.930} = 114.76$$

The value of F obtained, 4.04, fails to reach the 5 per cent point (4.38) for $n_1 = 1$ and $n_2 = 19$ degrees of freedom.

It is noted that in testing the significance of both the correlation coefficient r and the regression coefficient b, both were nonsignificant. The tests for significance of r and b are equivalent. When one is significant the other is significant also and vice versa. Exactly the same probabilities are obtained by the two tests of significance.

TABLE 53.—TESTING SIGNIFICANCE OF A REGRESSION COEFFICIENT

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Regression	1	114.76	114 76	4.04
Deviations from regression	19	539.53	28.40	
Total	20	654.29		

If the sum of squares due to regression is divided by the total sum of squares, we may express this as a percentage of the total sum of squares accounted for by regression. Such a quantity is r^2. The square of the correlation coefficient may be used as a measure of the percentage of the total variation accounted for. The correlation of +.419 indicated that 18 per cent of the variability in yield was accounted for by its association with number of kernels per spikelet. Little importance can be attached to this, however, since the correlation was not significant.

For prediction purposes, use may be made of the regression equation given by

$$Y = \bar{y} + b(x - \bar{x})$$

where Y = predicted yield.
$\bar{y}$ = observed mean yield.
x = number of kernels per spikelet.

Since $\bar{y} = 1619.3 \div 63 = 25.703$, $\bar{x} = 86.9 \div 63 = 1.3794$, and b for variety regression was +11.109,

$$Y = 25.70 + 11.109(x - 1.3794).$$

Multiplying -1.3794 by 11.109 and adding 25.70 gives

$$Y = 10.38 + 11.109x$$

From this equation, the predicted values of Y (yield per acre) can be calculated for varieties with different numbers of kernels per spikelet. A few such are calculated for illustration.

Variety	Average number of kernels per spikelet (x)	Yield per acre, bu.	
		Observed (y)	Predicted (Y)
Marquis	1.47	24.6	26.7
Ceres	1.73	30.5	29.6
Hope	1.13	22.2	22.9
Ceres × Hope No. 1	1.50	20.8	27.0

The observed and predicted yields are given merely as an example of procedure. Unless correlation or regression is significant and relatively high, it is apparent that prediction values will not be very accurate.

MEANS AND DIFFERENCES OF CORRELATION COEFFICIENTS

Frequently it is desired to determine the significance of a difference between two correlation coefficients. The method will be illustrated with the use of the interannual correlation coefficients for loaf volume determined from grain of spring-wheat varieties and strains grown in the regular rod-row nurseries in four places in the state, as given by Ausemus *et. al.* (1938).

Since correlation coefficients cannot be averaged directly, it is necessary to transform the coefficients to the statistic z (Fisher 1938) and test the significance of the difference between the z values by means of its error. The standard error of z is $1/\sqrt{N-3}$.

The computations for testing the significance of the difference between the interannual correlation coefficients for loaf volume in 1929–1930, determined from 25 varieties, and in 1931–1932, determined from 16 varieties, are carried through in Table 54.

TABLE 54.—TEST OF SIGNIFICANCE OF A DIFFERENCE BETWEEN CORRELATION COEFFICIENTS

Years correlated	Observed r	z	$N-3$	Reciprocal
1929–1930	+.43	.460	22	.0455
1931–1932	+.15	.151	13	.0769
		Difference = .309 ± .350,		Sum = .1224.

The observed values of r are first transformed to z with the use of Appendix Table IV. The two correlation coefficients were

based on 25 and 16 pairs of observations each; so $N - 3$ was 22 and 13, respectively. The sum of the reciprocals of $N - 3$ is the variance of the difference between the values of z. The square root of .1224 = .350 and is the standard error of the difference. The difference was less than its standard error and, therefore, it may be concluded that the two values of r were not significantly different.

When several correlation coefficients for the same characters are available, it frequently is desirable to determine the average correlation. This may be done by transformations of r to z, calculating the average value of z and then transforming the average z back to r. With the use of data from the same study by Ausemus *et al.*, where the correlation coefficients of +.81, +.43, and +.15 were based on 11, 25, and 16 determinations, respectively, the calculations are carried through in Table 55.

TABLE 55.—AVERAGING CORRELATION COEFFICIENTS

Years correlated	Observed r	z	$N - 3$	$(N - 3)z$
1927–1928	+ 81	1 127	8	9 016
1929–1930	+ 43	.460	22	10.120
1931–1932	+.15	.151	13	1.963
	+ 455	491	43	21.099

The values of r are first transformed to z, with the use of Appendix Table IV. Each value of z is multiplied by $N - 3$ and added to obtain 21.099. Dividing 21.099 by the sum of $N - 3$, or 43, gives .491 as the average value of z. This is then transformed to r by means of Appendix Table IV to give an average correlation coefficient of +.455. The standard error of r = +.455 will be $1/\sqrt{43}$ or .152. The accuracy of this average correlation is equivalent to a single test involving 43 + 3 = 46 pairs of observations. This average correlation is highly significant.

Before averaging correlation coefficients, it would be desirable to test whether they are homogeneous, *i.e.*, whether it can be assumed that they could have arisen from a population with the mean correlation coefficient through errors of random sampling.

The procedure in making such a test has been given by Rider (1939). The computations are carried through in Table 56, with the use of data given in Table 55.

TABLE 56.—TEST FOR HOMOGENEITY OF CORRELATION COEFFICIENTS

Years correlated	r	z	$N - 3$	$(N - 3)z$	$(N - 3)z^2$
1927–1928.....	+.81	1.127	8	9.016	10.161
1929–1930.....	+.43	0.460	22	10.120	4.655
1931–1932.....	+ 15	0.151	13	1.963	0.296
Sum.....	... .	....	43	21.099	15 112

Homogeneity of z can be tested by means of the χ^2 test, where

$$\chi^2 = S(N - 3)z^2 - \frac{[S(N - 3)z]^2}{S(N - 3)} = 15.112 - \frac{(21.099)^2}{43} = 4.759$$

for

$$k - 1 = 2 \text{ degrees of freedom,}$$

where k = number of correlation coefficients.

In this problem, χ^2 does not reach the 5 per cent point of 5.99 (Appendix Table III) for 2 degrees of freedom. It may be concluded that these three correlation coefficients could have come from equally correlated populations, the mean of which was found previously to be $r = +.455$.

PARTIAL CORRELATION

An extension of the idea of correlation leads to its application to groups of more than two variables. Partial- and multiple-correlation coefficients then become of considerable interest. Frequently two characters are related because of a third variable that affects both. By means of partial correlation, the relationship between two variables may be determined when the effect of other variables is eliminated.

High-yielding varieties of grain of high quality are two of the major objectives in crop improvement. Resistance to disease is of major importance, also, if the disease affects the yield or quality of the crop. In order to plan the breeding program, it is necessary that the plant breeder have a knowledge of the characters that are of greatest value under particular environmental conditions and the interrelationships between them. In determining the interrelationships between a number of characters, the method of partial correlation is useful in determining the relationship between two characters independent of the accompanying variation due to the other variables.

TABLE 57.—MEAN YIELD, PLUMPNESS OF KERNEL, DATE HEADING, AND CROWN-RUST PERCENTAGE IN ROD-ROW TRIALS WITH OATS

Variety or strain	Nursery stock number	Yield	Plump-ness	Date heading	Crown rust
Victory	514	36.5	3	7-11	14
Minota	512	38 0	9	7-11	17
Minota × White Russian	II-18-37	60.2	58	7-7	11
Black Mesdag	.....	40.2	13	7-3	65
Double Cross	II-22-35	36.3	17	7-8	30
Double Cross	II-22-36	40.0	15	7-6	38
Double Cross	II-22-37	51.8	43	7-6	25
Double Cross	II-22-38	57.3	28	7-7	10
Double Cross	II-22-39	40.6*	5	7-6	60
Double Cross	II-22-40	49.0	12	7-5	60
Double Cross	II-22-41	43.8	7	7-5	57
Double Cross	II-22-42	39.4	7	7-5	60
Double Cross	II-22-43	48.5	13	7-4	40
Double Cross	II-22-44	40.7	2	7-6	50
Double Cross	II-22-45	48.7	37	7-4	28
Double Cross	II-22-46	51.0	28	7-5	20
Double Cross	II-22-47	40.8	5	7-5	40
Double Cross	II-22-48	38.5	7	7-7	33
Double Cross	II-22-49	40.1	10	7-7	23
Double Cross	II-22-50	59.7	30	7-5	8
Double Cross	II-22-51	45.7	5	7-7	20
Double Cross	II-22-52	33.0	7	7-7	40
Double Cross	II-22-53	49 5	48	7-3	43
Double Cross	II-22-54	53.9	37	7-3	65
Double Cross	II-22-55	54.4*	50	7-3	63
Double Cross	II-22-56	37.2	32	7-4	50
Double Cross	II-22-57	40 5	25	7-5	38
Double Cross	II-22-58	48.8	32	7-3	60
Double Cross	II-22-59	47 6	15	7-4	53
Double Cross	II-22-60	51.1	23	7-4	56
Double Cross	II-22-61	53.4	23	7-4	53
Double Cross	II-22-62	55.9	52	7-4	27
Double Cross	II-22-63	54.9	55	7-4	18
Double Cross	II-22-64	46.2	15	7-3	47
Double Cross	II-22-65	49.3	10	7-3	30
Double Cross	II-22-66	46.4	35	7-3	33
Double Cross	II-22-67	54.4	23	7-4	35
Double Cross	II-22-68	52.1	23	7-5	20
Double Cross	II-22-69	70.5	67	7-3	15
Double Cross	II-22-70	72.9	67	7-3	25
Double Cross	II-22-71	21.2	7	7-7	40
Double Cross	II-22-72	24.6	0	7-10	30
Double Cross	II-22-73	53.2	57	7-3	37
Double Cross	II-22-74	50.9	17	7-5	37
Double Cross	II-22-75	61.7	30	7-5	15
Double Cross	II-22-76	53.4	12	7-7	12
Double Cross	II-22-77	43.1	22	7-4	25
Double Cross	II-22-78	54.7	13	7-6	15
Double Cross	II-22-79	57.2	47	7-5	7
Double Cross	II-22-80	38.8	10	7-4	37

* Grown in two plots.

An illustration of the methods of computation will be made with data collected at University Farm, St. Paul, Minnesota, from rod-row trials of oats, where three plots of each variety or strain were grown and the average of the three replications was used. Yield was expressed in bushels per acre, plumpness of grain was a visual note taken as a percentage, and the amount of crown rust was determined in percentage.

Plumpness of grain in small grains has been found to be directly correlated with yield. Frequently yield is associated with earliness. Both yield and plumpness of grain are influenced to a considerable extent by rust. By means of partial correlation, it was possible to determine the degree of association between yield and plumpness when the effect of differences in rust reaction was eliminated. Data to illustrate the computation of partial-correlation coefficients are given in Table 57.

For a more complete description of partial- and multiple-correlation methods, the reader may be referred to Wallace and Snedecor (1931).

In the methods to be given, the first step is the calculation of the simple, or total, correlation coefficients. For convenience of presentation, the following symbols will be used:

A = yield in bushel per acre.
B = plumpness of kernel.
C = date of heading.
D = percentage infection with crown rust.

The total correlation coefficients for all possible relationships between these four variables are given in Table 58.

TABLE 58.—TOTAL CORRELATION COEFFICIENTS FOR ALL INTERRELATIONSHIPS BETWEEN YIELD, PLUMPNESS, DATE OF HEADING, AND PERCENTAGE OF CROWN RUST

	A	B	C
B	+.7344*		
C	−.4898*	−.4968*	
D	−.3195†	−.2320	−.4012*

* Exceeds the 1 per cent level of significance.
† Exceeds the 5 per cent level of significance.

In this study, there were 50 pairs in the sample, and the degrees of freedom for testing the significance of a total-correlation coefficient would be $N - 2$ or 48. If Appendix Table V is

referred to, it is noted that all correlation coefficients except that between plumpness and crown-rust infection, $r_{BD} = -.2320$, exceeded the 5 per cent point and that all but this coefficient and $r_{AD} = -.3195$ exceeded the 1 per cent point.

A simple method of calculating partial-correlation coefficients will be illustrated in detail. The partial-correlation coefficients will be calculated from the *standard* partial-regression coefficients by utilizing the fact that $r_{12.34} = \sqrt{\beta_{12.34} \times \beta_{21.34}}$, where $r_{12.34}$ means the correlation between variables 1 and 2 with 3 and 4 eliminated and $\beta_{12.34}$ and $\beta_{21.34}$ are the standard regression coefficients. These regression coefficients are calculated by solving sets of normal equations as illustrated in Table 59.

TABLE 59.—SOLUTION OF NORMAL EQUATIONS TO OBTAIN STANDARD PARTIAL-REGRESSION COEFFICIENTS

	Line	*D*	*C*	*B*	*A*	Sum
Enter r_{DD} r_{DC}, r_{DB}, r_{DA} ..... *D*.	1	1.0000	− .4012	− .2320	− .3195	+ .0473
Change signs.....	2	−1.0000	+ .4012	+ .2320	+ .3195	
Enter r_{CC}, r_{CB}, r_{CA} ..... *C*.	3	...	1.0000	− .4968	− .4898	− .3878
Multiply line 1 by 2.*C*.....	4	...	− .1610	− .0931	− .1282	+ .0190
Add lines 3 and 4.....	5	..	+ .8390	− .5899	− .6180	− .3688
Divide line 5 by 5.*C*, and change signs.....	6	..	−1.0000	+ .7031	+ .7366	+ .4397✓
Enter r_{BB}, r_{BA}..... *B*.	7		..	1.0000	+ .7344	+1.0056
Multiply line 1 by 2.*B*	8			− .0538	− .0741	+ .0110
Multiply line 5 by 6.*B*.	9	..	. .	− .4148	− .4345	− .2593
Add lines 7, 8, and 9..	10	..	...	+ .5314	+ .2258	+ .7573
Divide line 10 by 10.*B*, and change signs.....	11	.. .	.....	−1.0000	− .4249	−1.4251✓
$\beta_{AB.CD}$ = +.4249	I			+ .4249	+ .4249	
$\beta_{AC.BD}$ = −.4379	II		− .4379	+ .2987	− .7366	
$\beta_{AD.BC}$ = −.3966	III	− .3966	− .1757	+ .0986	− .3195	

NOTE: In the instructions 2.*C* represents +.4012 in line 2, column *C*, etc.

First the correlation coefficients are entered in the table and added to obtain the sum. The correlation of $r_{DD} = 1$. To obtain the sum for line 3, in the table, add the three correlation coefficients in this line plus r_{CD}. The sum for line 7 is the sum of the total correlations in this line plus r_{BC} and r_{BD}. Proceed as directed in the instructions for each line, 1 to 11, in the table. The "sum" column serves as a check for all preceding work.

The figures marked $\surd$ must check, within rounding off of decimals, the sum of the figures in that line to the left of the sum column.

To calculate the partial-regression coefficients, bring down the figures in column A, lines 11, 6, and 2, in that order, and change signs to form I, II, and III, column A. Write figure in I.A one column to the left. This is $\beta_{AB.CD}$. Multiply I.B by 6.B and 2.B, and write down products under II.B and III.B, respectively. Thus, $(+.4249) \times (+.7031) = +.2987$ and $(+.4249) \times (+.2320) = +.0986$. Add II.$A$ and II.B to obtain II.C. This is the partial-regression coefficient $\beta_{AC.BD}$. Then multiply II.C by 2.C, or $(-.4379) \times (+.4012) = -.1757$, and record as III.$C$. Add III.$A$ + III.B + III.C to obtain III.D. This is the partial-regression coefficient $\beta_{AD.BC}$.

It is noted that in Table 59 the partial-regression coefficients with A as a dependent variable were determined. The rule is that the variable in the last column (if the column of sums is omitted) is the first term of the regression coefficients; the second term is that in the same vertical column. To obtain all possible partial-regression coefficients, each variable must, in turn, be placed in the last column and the set of normal equations solved anew. To save time, it is best to set up the columns so that the characters in the last two come in pairs, *i.e.*, D, C, B, A; D, C, A, B and A, B, D, C; A, B, C, D. By so doing, part of the computations from the first of a pair of characters can be copied off for the second.

Through such calculations, $\beta_{AB.CD} = +.4249$ and $\beta_{BA.CD} = +.5102$. The partial-correlation coefficient will be

$$r_{AB.CD} = \sqrt{\beta_{AB.CD} \times \beta_{BA.CD}}$$
$$= \sqrt{.4249 \times .5102} = +.4656$$

The significance of the partial-correlation coefficients may be determined by reference to Appendix Table V for $N - 4$ or 46 degrees of freedom. In general, the degrees of freedom are $N - p - 2$, where N is the number of observations and p is the number of variables eliminated. This amounts to the number of observations minus the number of variables.

The more interesting partial-correlation coefficients are given with the total-correlation coefficients for comparison.

$r_{AB} = +.7344^*$ $\quad r_{AB \cdot CD} = +.4656^*$
$r_{AC} = -.4898^*$ $\quad r_{AC \cdot BD} = -.4546^*$
$r_{AD} = -.3195\dagger$ $\quad r_{AD \cdot BC} = -.4600^*$

* Exceeds the 1 per cent point.
† Exceeds the 5 per cent point.

The partial correlation between yield and plumpness was highly significant even after the effects of date heading and amount of crown rust were eliminated. This strong relationship between yield and plumpness of grain may be made use of during the segregating generations in a breeding program when yield tests are not practical. Selection may be made for plumpness of grain, if it is recognized that plumpness is strongly associated with yield. The association between yield and date heading was of the same order of magnitude after plumpness and crown-rust differences were eliminated as when these were not. The partial correlation between yield and percentage of crown rust was highly significant when the effect of date heading and plumpness of seed was eliminated. Apparently crown rust significantly reduced yields apart from the influence of plumpness of grain and date of heading.

MULTIPLE CORRELATION

The multiple-correlation coefficient measures the degree to which the dependent variable is influenced by a series of other factors studied. It may be calculated from the total-correlation coefficients and the standard partial-regression coefficients. The formula is

$$R^2_{A \cdot BCD} = (r_{AB} \times \beta_{AB \cdot CD}) + (r_{AC} \times \beta_{AC \cdot BD}) + (r_{AD} \times \beta_{AD \cdot BC})$$

By substituting the values of r and β obtained in this problem

$$R^2_{A \cdot BCD} = (.7344 \times .4249) + (-.4898 \times -.4379) + (-.3195 \times -.3966) = .6532$$

$$R = .8082$$

The significance of a multiple-correlation coefficient may be tested by using Appendix Table V for $N - 4 = 46$ degrees of freedom and entering the column for four variables. The multiple-correlation of $R = .8082$ was highly significant. Squaring this correlation coefficient gives 65 per cent as the percentage of the variability in yield accounted for by its association with plumpness of grain, date of heading, and percentage of crown rust.

CHAPTER XXII

MULTIPLE EXPERIMENTS, METHODS FOR TESTING A LARGE NUMBER OF VARIETIES, AND THE ANALYSIS OF DATA EXPRESSED AS PERCENTAGES

MULTIPLE EXPERIMENTS IN RANDOMIZED BLOCKS

The same 10 varieties of barley as those used in Chap. XX to illustrate the computations for a randomized-block trial at University Farm were tested also in five other stations in Minnesota, namely, Waseca, Morris, Crookston, Grand Rapids, and Duluth. These tests were conducted in order to determine varietal adaptation in different regions of the state. Since studies of this nature will be of frequent occurrence in regional trials, it will be of interest to illustrate the methods of analysis that can be made.

In Table 60 is given the yields of 5 of the 10 varieties of barley in each of 3 randomized blocks at each of 4 stations for each of 2 years. Since the computations are designed only to illustrate the principles involved, the data are reduced from the more extended test actually made. The analysis of the data follows closely the method outlined by Immer, Hayes, and Powers (1934).

The degrees of freedom for an individual test at one location in 1 year would be keyed out as follows:

Variation Due to	Degrees of Freedom
Blocks	2
Varieties	4
Error (blocks × varieties)	8
Total	14

For the complete analysis of all data combined, the degrees of freedom could be keyed out as given in the summary on page 341.

The key to the degrees of freedom on page 341 illustrates the analogy between the individual tests and the complete analysis for all data combined. It is to be noted that the degrees of freedom for blocks within tests, varieties within tests, and error

TABLE 60.—YIELDS OF FIVE VARIETIES OF BARLEY, REPLICATED THREE TIMES IN EACH OF FOUR LOCATIONS IN 1932 AND 1935

Variety	Block number				Block number				Sum for both years
	I	II	III	Sum	I	II	III	Sum	
	University Farm, 1932				University Farm, 1935				
Manchuria	19.7	31.4	29 6	80.7	45 5	50 3	60.0	155.8	236.5
Glabron	28.6	38 3	43 5	110 4	47.5	41.1	49.4	138.0	248.4
Velvet	20 3	27.5	32.6	80 4	54.2	52.3	64.5	171 0	251.4
Barbless	27.9	40 0	46 1	114.0	62 2	53.1	74.7	190 0	304 0
Peatland	22.3	30.8	31 1	84.2	47 4	57 8	50.5	155.7	239.9
Sum	118.8	168 0	182.9	469.7	256.8	254 6	299 1	810 5	1280.2
	Waseca, 1932				Waseca, 1935				
Manchuria	40.8	29.4	30 2	100.4	53 9	58 8	47 7	160.4	260.8
Glabron	44 4	34.9	33.9	113.2	63.7	61.1	52.2	177 0	290.2
Velvet	44.6	41.4	26 2	112.2	53.9	59 1	56.4	169.4	281.6
Barbless	39 8	39 2	29 1	108 1	74.2	75 6	67.0	216.8	324 9
Peatland	71 5	47 6	55 4	174.5	51.1	47 3	45 0	143 4	317.9
Sum	241.1	192 5	174 8	608.4	296.8	301 9	268.3	867 0	1475.4
	Crookston, 1932				Crookston, 1935				
Manchuria	34.7	29.1	35.1	98.9	42.1	47.1	30 8	120.0	218.9
Glabron	28.8	28.7	21.0	78.5	38.8	29.4	30.5	98 7	172.2
Velvet	29 8	38 4	28.0	96 2	42.1	40.0	39.8	121.9	218.1
Barbless	27.7	27 6	20.4	75.7	44.3	43 5	47 7	135.5	211.2
Peatland	43 0	32 7	32.0	107.7	53.9	51 8	50 3	156 0	263.7
Sum	164.0	156.5	136 5	457.0	221.2	211 8	199.1	632.1	1089.1
	Grand Rapids, 1932				Grand Rapids, 1935				
Manchuria	20.2	30 2	16.0	66.4	26.6	26.5	32.7	85.8	152.2
Glabron	13 2	20.5	9.6	43 3	21.4	18 7	24.1	64.2	107.5
Velvet	24.5	41 6	30.6	96.7	20.7	26 8	30 4	77.9	174.6
Barbless	19.0	18.4	24 6	62.0	20.7	23 6	30.9	75.2	137.2
Peatland	27 6	30.0	22 7	80.3	32.6	40.0	34 2	106.8	187.1
Sum	104.5	140.7	103.5	348.7	122.0	135.6	152 3	409.9	758.6
Sum of 4 stations	628.4	657.7	597.7	1883.8	896.8	903.9	918.8	2719.5	4603.3

are the product of the degrees of freedom in a single test multiplied by the number of tests.

Variation Due to	Degrees of Freedom
Between tests	7
Stations	3
Years	1
Stations × years	3
Between blocks within tests	16
Blocks	2
Blocks × stations	6
Blocks × years	2
Blocks × stations × years	6
Between varieties within tests	32
Varieties	4
Varieties × stations	12
Varieties × years	4
Varieties × stations × years	12
Error within tests	64
Blocks × varieties	8
Blocks × varieties × stations	24
Blocks × varieties × years	8
Blocks × varieties × stations × years	24
Total	119

The degrees of freedom for the main effects, such as stations, years, blocks, and varieties, are 1 less than the number of stations, years, blocks, or varieties, respectively. The degrees of freedom for the interactions are the product of the degrees of freedom for the main effects involved.

Once the degrees of freedom are keyed out, the calculation of the sums of squares must be made in accordance with this plan. Before proceeding with the complete analysis, it will be well to test the errors of the eight separate tests for homogeneity in order to determine whether they may legitimately be combined into a single analysis with a single error. In Table 61 are given the sums of squares calculated separately for each of the eight tests.

The χ^2 (Chi-square) distribution can be used as an approximate test of the homogeneity of several estimates of variance. The method proposed by Bartlett (1937) will be used to determine whether the variances calculated for the separate tests can be considered homogeneous, *i.e.*, whether they can be considered random sampling deviates from the mean of these variances.

The formula for χ^2 will be $\chi^2 = \frac{1}{C}\{n \log_e s^2 - S(n_r \log_e s_r^2)\}$ for $k - 1$ degrees of freedom, where k is the number of variances being compared, n_r is the degrees of freedom of each variance, n is the total degrees of freedom for the separate variances and

TABLE 61.—SUMS OF SQUARES CALCULATED FOR THE SEPARATE TESTS

Station and year of test	Sums of squares for			
	Total	Blocks	Varieties	Error
University Farm, 1932	867 30	450 10	375.61	41 59
University Farm, 1935	1031 71	251 62	506 36	273 73
Waseca, 1932	1907 14	471.40	1196 33	239 41
Waseca, 1935	1203.56	131 15	993.84	78.57
Crookston, 1932	487 87	80 83	252 62	154 42
Crookston, 1935	807 84	49 21	595.82	162.81
Grand Rapids, 1932	905 56	179.69	536.17	189.70
Grand Rapids, 1935	509 91	92 13	336 46	81.32
Sum	7720 89	1706 13	4793 21	1221.55

TABLE 62.—CALCULATION OF χ^2 TEST FOR HOMOGENEITY OF VARIANCES

Station and year of test	Degrees of freedom in each test, n_r	Error variance of each test, s_r^2	$\log_e s_r^2$	$n_r s_r^2$	$n_r \log_e s_r^2$
University Farm, 1932	8	5.20	1 6487		
University Farm, 1935	8	34 22	3 5328		
Waseca, 1932	8	29.93	3.3989		
Waseca, 1935	8	9.82	2 2844		
Crookston, 1932	8	19.30	2 9601		
Crookston, 1935	8	20.35	3.0131		
Grand Rapids, 1932	8	23.71	3 1659		
Grand Rapids, 1935	8	10 16	2 3184		
Sum	64	152.69	22.3223	1221.52	178.5784

$S(n_r)$, s_r^2 refers to the individual variances, s^2 the pooled variance calculated from $S(n_r s_r^2)/n$, and C is a correction term defined by

$$C = 1 + \frac{1}{3(k-1)}\left\{S\left(\frac{1}{n_r}\right) - \frac{1}{n}\right\}$$

The estimated variances will be found by dividing the error sums of squares in Table 61 by 8 degrees of freedom. Table 62 may be formed to illustrate the computations.

Since the degrees of freedom are the same for each test, the sum of the products $S(n_r s_r^2)$, and $S(n_r \log_e s_r^2)$ may be calculated from the totals of columns 2, 3, and 4 in Table 62; otherwise each value of $n_r s_r^2$ and $n_r \log_e s_r^2$ would need to be calculated and the column added to obtain the sum. In this problem $S(n_r s_r^2) = 1221.52$ is found by multiplying 152.69 by 8 and $S(n_r \log_e s_r^2) = 178.5784$ is obtained by multiplying 22.3223 by 8. Since

$$n = 64$$

$$s^2 = \frac{1221.52}{64} = 19.086$$

$$n \log_e s^2 = 64 \times 2.94891 = 188.7302$$

$$C = 1 + \tfrac{1}{21}\{(\tfrac{1}{8} + \tfrac{1}{8} + \tfrac{1}{8} + \tfrac{1}{8} + \tfrac{1}{8} + \tfrac{1}{8} + \tfrac{1}{8} + \tfrac{1}{8}) - \tfrac{1}{64}\} = 1.0469$$

$$\chi^2 = \frac{188.7302 - 178.5784}{1.0469} = 9.70, \text{ for 7 degrees of freedom.}$$

Referring to the table of χ^2 (Appendix Table III) for 7 degrees of freedom, we find that $\chi^2 = 9.80$ when $P = .20$. We may conclude, therefore, that deviations between variances as great as these observed would occur more than 20 times in 100 through errors of random sampling. These 8 error variances may, therefore, be considered homogeneous, and it will be legitimate to replace the 8 separate variances by their mean variance (19.09) in the analysis of variance of all data.

The total yield of the 120 plots, as given in Table 60, was 4603.3 bu. The correction term $S(x)\bar{x} = \frac{[S(x)]^2}{N} = \frac{(4603.3)^2}{120} =$ 176,586.42. To obtain the total sum of squares, the squares of the individual plots are added to give $S(x)^2 = 200{,}879.35$, and the correction term $S(x)\bar{x}$ is subtracted to give 24,292.93.

Several other tables need to be set up by adding the appropriate yields in Table 60. In Table 63 are given the total yields for each variety at each station by adding the yields for both years.

The figures in Table 63 are taken directly from the right-hand margin of Table 60. They are assembled here for convenience, with the appropriate variety and station totals.

In Table 64 are given the data for comparisons of varieties in different years, obtained by adding the yields at the four stations.

TABLE 63.—TOTAL YIELDS GROUPED FOR VARIETIES AND STATIONS

Variety	Station				Sum
	University Farm	Waseca	Crookston	Grand Rapids	
Manchuria	236.5	260.8	218 9	152 2	868.4
Glabron	248.4	290.2	177 2	107.5	823.3
Velvet	251.4	281 6	218.1	174.6	925.7
Barbless	304.0	324.9	211.2	137.2	977 3
Peatland	239 9	317 9	263 7	187 1	1008 6
Sum	1280 2	1475 4	1089 1	758 6	4603 3

TABLE 64.—TOTAL YIELDS GROUPED FOR VARIETIES AND YEARS

Variety	Year		Sum
	1932	1935	
Manchuria	346 4	522.0	868.4
Glabron	345 4	477.9	823 3
Velvet	385 5	540.2	925.7
Barbless	359 8	617.5	977 3
Peatland	446 7	561.9	1008 6
Sum	1883 8	2719.5	4603 3

In Table 65 are assembled the data for comparisons of blocks and stations, obtained by adding the block totals for the 2 years of each station.

TABLE 65.—TOTAL YIELDS OF BLOCKS AND STATIONS

Block	Station				Sum
	University Farm	Waseca	Crookston	Grand Rapids	
I	375.6	537.9	385.2	226 5	1525.2
II	422.6	494.4	368 3	276.3	1561.6
III	482.0	443.1	335.6	255.8	1516.5
Sum	1280.2	1475 4	1089 1	758.6	4603.3

In Table 66 are the totals for comparison of blocks and years. This table is assembled from the totals at the bottom of Table 60.

TABLE 66.—TOTAL YIELDS OF BLOCKS AND YEARS

Block	Year		Sum
	1932	1935	
I	628.4	896.8	1525.2
II	657.7	903.9	1561 6
III	597 7	918 8	1516.5
Sum	1883.8	2719.5	4603 3

One other table is necessary, that of stations and years. This is given as Table 67. The figures for this comparison are assembled here for convenience, also, but could have been found directly in Table 60.

TABLE 67.—TOTAL YIELDS OF STATIONS AND YEARS

Year	Station				Sum
	University Farm	Waseca	Crookston	Grand Rapids	
1932	469.7	608.4	457.0	348.7	1883.8
1935	810.5	867.0	632.1	409 9	2719.5
Sum	1280.2	1475.4	1089.1	758.6	4603 3

The calculation of the sums of squares for the complete analysis can be performed with the least difficulty and confusion if the steps are carried through in a routine manner. Many of the calculations are given in Table 68. The remainder follow easily and logically.

In Table 68, x is used to designate the individual plots and x_s, x_y, x_v, and x_b, the total yields for each station, year, variety, and block, respectively. The symbols x_{vs}, x_{vy}, etc., refer to the totals for each variety at each station, each variety each year, etc., as found within Tables 63 to 67. The symbol x_{vsy} refers to the total yield of each variety at each station for a single year.

Column 2 of Table 68 gives the number of figures squared in calculating column 1, and column 3 gives the number of plots

in each figure squared. Column 4 is necessary to reduce the sums of squares to a single-plot basis. Column 6 gives the sums of squares, and column 7 the degrees of freedom.

TABLE 68.—CALCULATION OF SUMS OF SQUARES

Variate	Total of squares (1)	Number of figures squared (2)	Number of plots in each figure squared (3)	(4 = 1 ÷ 3)	Correction term $S(x)\bar{x}$ (5)	Sum of squares (6 = 4 − 5)	Degrees of freedom (7)
$S(x^2)$	200,879.35	120	1	200,879.35	176,586.42	24,242.93	119
$S(x_s^2)$	5,577,329.97	4	30	185,911.00	176,586.42	9,324.58	3
$S(x_y^2)$	10,944,382.69	2	60	182,406.38	176,586.42	5,819.96	1
$S(x_v^2)$	4,261,251.19	5	24	177,552.13	176,586.42	965.71	4
$S(x_b^2)$	7,064,601.85	3	40	176,615.05	176,586.42	28.63	2
$S(x_{vs}^2)$	1,129,020.73	20	6	188,170.12	176,586.42	11,583.70	19
$S(x_{vy}^2)$	2,206,627.61	10	12	183,885.63	176,586.42	7,299.21	9
$S(x_{bs}^2)$	1,871,824.37	12	10	187,182.44	176,586.42	10,596.02	11
$S(x_{by}^2)$	3,650,180.03	6	20	182,509.00	176,586.42	5,922.58	5
$S(x_{sy}^2)$	2,897,377.01	8	15	193,158.47	176,586.42	16,572.05	7
$S(x_{bsy}^2)$	974,322.93	24	5	194,864.59	176,586.42	18,278.17	23
$S(x_{vsy}^2)$	593,855.03	40	3	197,951.68	176,586.42	21,365.26	39

The sums of squares for the first-order interactions are obtained by subtracting from the sum of squares for the two variables in Table 68 the sums of squares for the two main effects. For example, the sum of squares for the interaction of varieties × stations will be given by the sum of squares for x_{vs}^2 in Table 68 minus the sum of squares for the main effects of varieties and stations x_v^2 and x_s^2. Numerically, this will be:

Sum of Squares	Degrees of Freedom
11,583.70	19
− 965.71	4 (varieties)
− 9,324.58	3 (stations)
1,293 41	12 (varieties × stations)

The second-order interaction of varieties × stations × years, for example, is obtained by subtracting from the sum of squares opposite $S(x_{vsy}^2)$ in Table 68 the sums of squares for varieties, stations, years, and all possible first-order interactions. Thus:

Sum of Squares	Degrees of Freedom
21,365.26	39
— 965.71	4 (varieties)
— 9,324.58	3 (stations)
— 5,819.96	1 (years)
— 1,293.41	12 (varieties × stations)
— 513 54	4 (varieties × years)
— 1,427.51	3 (stations × years)
2,020.55	12 (varieties × stations × years)

The complete analysis of variance is now carried through in Table 69, the error sum of squares being obtained as a remainder.

TABLE 69.—COMPLETE ANALYSIS OF VARIANCE

Variation due to	Degrees of freedom	Sum of squares	Mean square	s	F
Stations	3	9,324 58	3108 19	....	162.82*
Years	1	5,819.96	5819 96	....	304.87*
Stations × years	3	1,427 51	475.84	...	24.93*
Blocks	2	28.63	14 32		
Blocks × stations	6	1,242 81	207 14	....	10 85*
Blocks × years	2	73 99	37.00	....	1 94
Blocks × stations × years	6	360.69	60 12	....	3.15*
Varieties	4	965 71	241 43	....	12.65*
Varieties × stations	12	1,293 41	107.78	....	5.65*
Varieties × years	4	513 54	128 39	....	6.73*
Varieties × stations × years.	12	2,020 55	168 38	...	8.82*
Error	64	1,221 55	19.09	4.37	
Total	119	24,292.93			

* Exceeds the 1 per cent level of significance when compared with error mean square.

The structure of the complete analysis of variance becomes clear when it is compared with the separate analyses of variance of the single tests. The sum of squares for error in Table 69 is the same as for the sum of all tests calculated separately in Table 61. The error mean square in the complete analysis is, then, the average of the eight individual error mean squares calculated separately in Table 62. Thus, $152.69 \div 8 = 19.09$, the mean square for error given in Table 62. Adding the sum of squares for varieties, varieties × stations, varieties × years, and varieties × stations × years in Table 69 gives 4793.21. This agrees with 4793.21 obtained as the sum of the sums of squares for varieties within tests given in Table 61. A similar comparison holds for blocks.

The manner in which the data can be interpreted will now be illustrated. From Table 69, it is seen that the mean square for varieties, varieties × stations, varieties × years, and varieties × stations × years, compared with error mean square, exceeded the 1 per cent point. It is plain, therefore, that there were significant differences in average yielding ability and that some varieties reacted in a differential manner at some stations and in some years.

A summary of the mean yields of the five varieties for 1932 and 1935 at each of the four stations is given in Table 70. The varieties are listed in the order of average yield at all stations.

TABLE 70.—MEAN YIELD OF FIVE VARIETIES OF BARLEY FOR 1932 AND 1935 AT EACH OF FOUR STATIONS AND THEIR AVERAGE YIELD AT ALL STATIONS

Variety	Station				Average
	University Farm	Waseca	Crookston	Grand Rapids	
Peatland	40.0	53.0	44.0	31.2	42.0
Barbless	50.7	54.2	35.2	22.9	40.7
Velvet	41.9	46.9	36.4	29.1	38.6
Manchuria	39.4	43.5	36.5	25.4	36.2
Glabron	41.4	48.4	29.5	17.9	34.3

The standard error of a single plot (Table 69) was 4.37 bu. Since 24 plots were involved in the variety averages for all stations and both years, the standard error of the mean of 24 plots would be $4.37/\sqrt{24} = 0.89$ bu., and the standard error of a difference between two variety means would be $0.89\sqrt{2} = 1.26$ bu.

A formula frequently used to obtain the standard error of an average of several tests is $1/N\sqrt{s_A^2 + s_B^2 + s_C^2 + \cdots}$, where N is the number of tests and s_A^2, s_B^2, etc., are the variances for error of the separate tests. If the variances of the mean of three plots were calculated for each of the eight tests by dividing each of the error variances, given as s_r^2 in Table 62, by 3, the standard error of the average of all tests would be

$$\frac{1}{8}\sqrt{\frac{5.20 + 34.22 + \cdots 10.16}{3}} = 0.89 \text{ bu.},$$

the same as calculated in the preceding paragraph.

With 64 degrees of freedom for error, $t = 2$ at the 5 per cent level of significance. If twice the standard error of the difference between two means as a minimum level of significance is accepted, it may be said that differences in excess of $2 \times 1.26 = 2.52$ bu. would be judged as probably significant. On this basis, Velvet, Manchuria, and Glabron would be significantly lower in yield than Peatland. Manchuria and Glabron would be significantly lower in yield than Barbless. Manchuria and Glabron were lowest in yield, and the difference between them was not significant.

The mean square for varieties × stations was significantly greater than the mean square for error. It is apparent that some varieties reacted in a differential manner at certain stations. A first-order interaction involves the difference between two differences. The mean yield of Barbless at University Farm, for an average of both years, exceeded that of Peatland by $50.7 - 40.0 = 10.7$ bu. per acre. At Grand Rapids, however, the difference between these two varieties was $31.2 - 22.9 = 8.3$ bu. in favor of Peatland. The question arises whether these two differences are significantly different. This difference between two differences will be $(50.7 - 40.0) - (22.9 - 31.2) = 19.0$ bu. Since six plots were involved in each mean being compared, the standard error of the cross difference will be $\frac{4.37}{\sqrt{6}} \sqrt{2} \sqrt{2} = 3.57$ bu. Twice this is 7.14 bu., and any "cross difference" exceeding this value is expected to occur less than once in 20 trials by random sampling alone. It is clear, therefore, that the yields of the varieties Barbless and Peatland were differential at University Farm and Grand Rapids. Other differential responses can be found also in Table 70. Extensive testing of these and other standard varieties of barley in Minnesota for a long period of years has shown Peatland to be better adapted at Grand Rapids than at any other experiment station in the state. From data such as these, carried out at six stations in the state for a minimum period of 3 years, general recommendations regarding varieties are made to the farmers. In many instances, significant interactions of varieties and stations are obtained, and certain varieties are recommended only for certain regions of the state.

Significant interactions of some varieties in the 2 years could be found by application of the general procedure outlined above.

Interactions of varieties × years are of less interest to the plant breeder than interactions of varieties × stations.

Although the second-order interaction of varieties × stations × years was significant also, this is of minor interest to the plant breeder. A significant second-order interaction means that certain differential responses of two varieties at each of two stations was not the same in each of 2 years.

For a complete understanding of an analysis of variance, of which that given in Table 69 is an example, one further comparison can be set up. Letting V, S, and Y represent variances due to varieties, stations, and years, respectively, and $V \times S$, $V \times Y$, and $V \times S \times Y$ the interaction variances, we may determine whether variance due to

$$\left.\begin{matrix} V > V \times S > V \times S \times Y \\ V > V \times Y > V \times S \times Y \end{matrix}\right\} \text{error}$$

by means of the F test. The symbol $>$ means "greater than." If variance due to varieties significantly exceeds the interaction of varieties × stations, we have evidence that varietal performance generally was consistent enough to demonstrate that some varieties were the best in all stations, as an average of the years in which tests were made. If the variety variance significantly exceeds that of varieties × years, we may conclude that as an average of all stations some varieties were consistently better in yield in all years.

Further, if the interaction of varieties × stations significantly exceeds varieties × stations × years, it is plain that the differential responses of the varieties at the separate stations were sufficiently similar in the different years to warrant the conclusion that these differential responses may be permanent features of these localities.

Unless the variance for varieties significantly exceeds that of varieties × stations, no general recommendation of a variety for the entire state can be made. Extensive tests in the region in which the varieties may be grown provide the only sound basis for recommendation over wide areas.

COMPARISON OF VARIETIES IN DIFFERENT EXPERIMENTS, WHERE THE SAME CHECK VARIETIES ARE GROWN

Frequently it may be desirable to compare the yields of varieties grown in different experiments. If the same check varieties

have been included in the different experiments, comparisons of the new varieties may be made by comparing them through the checks. Thus, if A and B are the yields of two varieties in different experiments and the same check (ch) has been included in each test, the relative difference in yield between A and B will be given by $(A - ch_1) - (B - ch_2)$, where ch_1 and ch_2 are the yields of the checks in the experiments involving A and B, respectively.

The standard error of the foregoing difference will be $\sqrt{2s_1^2 + 2s_2^2}$, where s_1^2 and s_2^2 are the variance of the mean for the two tests.

If more than one check variety has been included in each test, comparisons may be made of $(A - \overline{ch}_1) - (B - \overline{ch}_2)$, where $\overline{ch}_1$ and $\overline{ch}_2$ are the means of the several checks. The standard error of this difference will be

$$\sqrt{\left(s_1^2 + \frac{s_1^2}{N}\right) + \left(s_2^2 + \frac{s_2^2}{N}\right)}$$

where s_1^2 and s_1^2 = variance of the mean for a single variety in experiments 1 and 2.

N = number of checks used in each experiment.

SIMPLE LATTICE EXPERIMENTS

When the number of varieties to be tested is small, the randomized block or Latin-square designs provide an efficient method for testing the significance of varietal differences. As the number of varieties becomes large, the randomized-block design with all varieties in the same block becomes less efficient because of increasing soil heterogeneity within blocks. The Latin-square design for large numbers of varieties cannot be used because of the prohibitive number of replications required.

Yates (1936) suggested a modification of the complete block design whereby the number of varieties in a block was less than the total number to be tested. The error could then be calculated from the variation within the small, or incomplete, blocks and would be lower, usually, than the error calculated from randomized complete blocks. The methods of analysis appropriate for such designs have been given by Yates (1936) and Goulden (1937, 1939). In these analyses some information about varietal difference was lost. As a result, these incomplete block designs

could be less efficient than ordinary randomized complete blocks if the soil were relatively homogeneous. Recently Yates (1939) and Cox, Eckhardt, and Cochran (1940) have shown how all the information regarding differences between varieties in different incomplete blocks could be recovered. As a consequence, these designs can never be appreciably less efficient than ordinary randomized blocks containing the total number of varieties and will be considerably more accurate if there is a reduction in the error through the use of the small, or incomplete, blocks.

TABLE 71.—RANDOM ARRANGEMENT OF VARIETIES IN LATTICE EXPERIMENT

Replication 1 (Group *X*)

Block					
(1)	10	7	6	8	9
(2)	14	13	11	15	12
(3)	2	4	5	3	1
(4)	25	24	23	21	22
(5)	18	16	17	20	19

Replication 2 (Group *Y*)

Block					
(6)	15	5	10	20	25
(7)	16	6	21	11	1
(8)	2	17	7	22	12
(9)	23	3	13	18	8
(10)	24	4	14	19	9

Replication 3 (Group *X*)

Block					
(11)	8	9	10	6	7
(12)	23	21	24	25	22
(13)	12	14	11	13	15
(14)	16	19	20	17	18
(15)	3	4	5	1	2

Replication 4 (Group *Y*)

Block					
(16)	13	8	3	23	18
(17)	2	22	12	17	7
(18)	19	14	9	24	4
(19)	21	11	16	1	6
(20)	10	15	20	5	25

The design and computation of the data for a lattice[1] experiment will be illustrated using uniformity trial data given by Wiebe (1935). It was assumed that 25 varieties grown in three-row plots and the central row harvested were to be tested, with four replications. The method of computation will follow closely that given by Cox, Eckhardt, and Cochran (1940).

In the lattice experiments described here, the number of varieties is a perfect square. The number of varieties, $v = k^2$, are tested in incomplete blocks of k varieties each. The varieties

[1] Certain of the lattice designs have been referred to as pseudofactorial arrangements in two equal groups of sets, as two-dimensional pseudofactorial arrangements with two equal groups of sets, and as two-dimensional quasi-factorial designs in randomized blocks in two equal groups of sets.

may be identified by numbers arranged in a square, as follows, with the use of $k^2 = 25$ varieties:

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25

TABLE 72.—YIELDS OF VARIETIES IN GRAMS PER ROD ROW

Replication 1 (Group X)

Block						Block totals
(3)	1 635	2 525	3 555	4 650	5 635	3,000
(1)	6 495	7 730	8 810	9 775	10 710	3,520
(2)	11 630	12 600	13 645	14 635	15 645	3,155
(5)	16 735	17 690	18 840	19 855	20 805	3,925
(4)	21 620	22 795	23 590	24 660	25 615	3,280
						16,880

Replication 2 (Group Y)

Block						Block totals
(7)	1 530	6 490	11 595	16 495	21 540	2,650
(8)	2 610	7 660	12 620	17 695	22 570	3,155
(9)	3 705	8 850	13 675	18 685	23 640	3,555
(10)	4 840	9 905	14 785	19 860	24 875	4,265
(6)	5 670	10 455	15 655	20 665	25 615	3,060
						16,685

TABLE 72.—YIELDS OF VARIETIES IN GRAMS PER ROD ROW.—(*Continued*)

Replication 3 (Group *X*)

Block						Block totals
(15)	1 635	2 700	3 640	4 640	5 645	3,260
(11)	6 570	7 545	8 675	9 580	10 470	2,840
(13)	11 550	12 515	13 450	14 550	15 495	2,560
(14)	16 505	17 620	18 700	19 570	20 575	2,970
(12)	21 445	22 455	23 445	24 465	25 515	2,325
						13,955

Replication 4 (Group *Y*)

Block						Block totals
(19)	1 550	6 655	11 545	16 515	21 550	2,815
(17)	2 455	7 425	12 460	17 470	22 440	2,250
(16)	3 445	8 545	13 700	18 530	23 525	2,745
(18)	4 510	9 525	14 515	19 395	24 425	2,370
(20)	5 540	10 575	15 610	20 510	25 615	2,850
						13,030

These 25 varieties are arranged in incomplete blocks of $k = 5$ varieties each. In one group, designated as *X*, the 5 varieties for each block are taken from a row of the foregoing square. In a second group, designated *Y*, the varieties are taken from a column of the foregoing square. The order of the 5 rows, or columns, is randomized, and the 5 varieties within each row or column are

TABLE 73.—COMBINATION OF REPLICATIONS
Group *X* (Replication 1 + Replication 3)

						Row totals
	1 1270	2 1225	3 1195	4 1290	5 1280	6,260
	6 1065	7 1275	8 1485	9 1355	10 1180	6,360
	11 1180	12 1115	13 1095	14 1185	15 1140	5,715
	16 1240	17 1310	18 1540	19 1425	20 1380	6,895
	21 1065	22 1250	23 1035	24 1125	25 1130	5,605
Column totals.	5820	6175	6350	6380	6110	30,835

Group *Y* (Replication 2 + Replication 4)

						Row totals
	1 1080	6 1145	11 1140	16 1010	21 1090	5,465
	2 1065	7 1085	12 1080	17 1165	22 1010	5,405
	3 1150	8 1395	13 1375	18 1215	23 1165	6,300
	4 1350	9 1430	14 1300	19 1255	24 1300	6,635
	5 1210	10 1030	15 1265	20 1175	25 1230	5,910
Column totals	5855	6085	6160	5820	5795	29,715

placed in random order. In Table 71 on page 352 is given the random arrangement of the 25 varieties in each of two replications of the *X* and *Y* groups.

Varieties 1, 2, 3, 4, and 5 fell in block 3 and again in block 15, both in group *X*. Varieties 1, 6, 11, 16, and 21 fell in blocks 7 and 19 in group *Y*. All 25 varieties are contained in each complete replication.

The foregoing random arrangement of varieties was superimposed on the plot yields, in grams per rod row, of Wiebe's (1935) data. The plot yields of each variety are given in Table 72, assembled according to rows and columns of the original square. The variety number is given above each plot yield.

The data from both replications of groups X and Y, for each variety, are added next and are given in Table 73.

The sums of the yields of the two plots each for the X and Y groups are combined next to give the total yields of the four plots of each variety. These total yields of the varieties are given in Table 74, with appropriate row and column totals.

TABLE 74.—TOTAL YIELDS OF VARIETIES

						Row totals
	1 2,350	2 2,290	3 2,345	4 2,640	5 2,490	12,115
	6 2,210	7 2,360	8 2,880	9 2,785	10 2,210	12,445
	11 2,320	12 2,195	13 2,470	14 2,485	15 2,405	11,875
	16 2,250	17 2,475	18 2,755	19 2,680	20 2,555	12,715
	21 2,155	22 2,260	23 2,200	24 2,425	25 2,360	11,400
Column totals	11,285	11,580	12,650	13,015	12,020	60,550

The total sum of squares is found by adding the squares of the 100 plot yields in Table 72 to give 38,012,350 and subtracting the correction term $(60,550)^2/100 = 36,663,025$ to give 1,349,325 as the total sum of squares.

The sum of squares for replications is calculated from the totals of the four complete replications, *i.e.*,

$$\frac{(16,880)^2 + (16,685)^2 + (13,955)^2 + (13,030)^2}{25} - 36,663,025$$

$$= 450,837$$

The sum of squares for varieties (ignoring blocks) is calculated from the variety totals in Table 74. Thus

$$\frac{(2350)^2 + (2290)^2 + \cdots + (2360)^2}{4} - 36,663,025 = 240,663$$

The sum of squares for blocks (eliminating varieties) is made up of two components as follows:

Component a is calculated from the sum of squares of differences between blocks containing identical varieties. These differences are found by subtracting the block totals for the same group of varieties in replications 1 and 3 and in 2 and 4, taken from Table 72. These differences are given below.

Set X			Set Y		
Replication 1	Replication 3	Difference	Replication 2	Replication 4	Difference
3,000	3,260	− 260	2,650	2,815	− 165
3,520	2,840	680	3,155	2,250	905
3,155	2,560	595	3,555	2,745	810
3,925	2,970	955	4,265	2,370	1895
3,280	2,325	955	3,060	2,850	210
16,880	13,955	2925	16,685	13,030	3655

The sum of squares of the deviations within these two sets of differences will give the sum of squares between paired blocks within sets. Thus

$$\frac{(-260)^2 + \cdots + (955)^2 + (-165)^2 + \cdots + (210)^2}{10} - \frac{(2925)^2 + (3655)^2}{50} = 346{,}262$$

The divisors are $2k = 10$ and $2k^2 = 50$.[1]

Component b is obtained from two sets of differences giving estimates of block yields freed of varietal effects. In Table 73,

[1] For six replications, there would be three columns of block totals for each set. Component a would then be calculated from an analysis of variance for each set, the degrees of freedom for set X being as follows:

	Degrees of freedom
Replications	2
Set X totals	$k - 1$
Interaction, or component (a)	$2(k - 1)$

The same would be done for set Y, and the degrees of freedom and sums of squares of both added to give the complete component a.

the row totals are the sums of two blocks of the same group of varieties. These row totals cannot be used to calculate block sum of squares, since they are confounded with (contain) varietal effects as well. The first row total in group X (Table 73), made up of the sums of the two blocks containing varieties 1, 2, 3, 4, and 5, comes to 6260. The first column total in group Y is 5855 and is clearly an estimate of the varietal effects alone of the same five varieties, since each block in group Y is equally represented. The difference between these totals, $6260 - 5855 = 405$, is an estimate of block effect freed of varietal differences. In a similar manner, the first row total in group Y (Table 73) minus the first column total in group X, $5465 - 5820 = -355$, is an estimate of block effect for blocks containing varieties 1, 6, 11, 16, and 21.

Since, however, it is easier to add than subtract in making adjustments to the average yields of the varieties, it is preferable to *subtract* the unconfounded from the confounded totals and work with the negative values. Thus, $5855 - 6260 = -405$ and $5820 - 5465 = 355$. These differences are designated rkc_x and rkc_y, where r is the number of replications and k the number of plots per block and c_x and c_y the mean corrections from the X and Y groups, respectively. The rkc_x values may be determined also by subtracting from the row totals of Table 74 twice the row totals of group X in Table 73. Thus, $12{,}115 - 2(6260) = -405$. The rkc_y values are obtained by subtracting from the column totals in Table 74 twice the row totals for group Y in Table 73. These values are given below:

rkc_x		rkc_y	
12,115 − 2(6260) =	− 405	11,285 − 2(5465) =	355
12,445 − 2(6360) =	− 275	11,580 − 2(5405) =	770
11,875 − 2(5715) =	445	12,650 − 2(6300) =	50
12,715 − 2(6895) =	−1075	13,015 − 2(6635) =	−255
11,400 − 2(5605) =	190	12,020 − 2(5910) =	200
	−1120		1120

The sum of the rkc_x and rkc_y values will be 0.

The sum of squares of deviations within sets of rkc_x and rkc_y will be an estimate of variance between blocks (eliminating varieties). Thus

$$\frac{(-405)^2 + \cdots + (190)^2 + (355)^2 + \cdots + (200)^2}{20} - \frac{(-1120)^2 + (1120)^2}{100} = 97{,}705$$

The divisors will be $rk = 20$ and $rk^2 = 100$.

The analysis of variance table may now be set up. This is given in Table 75.

TABLE 75.—ANALYSIS OF VARIANCE OF LATTICE EXPERIMENT

Variation due to	Degrees of freedom		Sum of squares		Mean square
Replications	..	3	.....	450,837	150,279 00
Component a	8	..	346,262	.....	43,282.75
Component b	8	..	97,705	.....	12,213.12
Blocks (eliminating varieties)	..	16	.....	443,967	27,747.94
Varieties (ignoring blocks)	..	24	.....	240,663	10,027.62
Error (intrablock)	..	56	.....	213,858	3,818.89
Total	..	99	.....	1,349,325	

A test of significance of variety mean square in the form of an F test cannot be made from the mean squares for varieties and error in Table 75, since variety mean square is partially confounded, *i.e.*, it contains some of the differences between blocks. Usually it will be sufficient to apply the test of significance appropriate for an ordinary randomized-complete-block test. Although less precise than the exact test, this usually will be adequate. A lattice experiment can always be analyzed as an ordinary randomized-complete-block test. Such a test is given in Table 76.

TABLE 76.—ANALYSIS OF VARIANCE AS RANDOMIZED COMPLETE BLOCKS

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Replications	3	450,837	150,279.00	
Varieties	24	240,663	10,027.62	1.10
Error	72	657,825	9,136.46	
Total	99	1,349,325		

In the foregoing table, the degrees of freedom and sums of squares for replications, varieties, and total are taken directly from Table 75 and the error degrees of freedom and sum of squares obtained by subtraction. In this case, $F = 10{,}027.62 \div 9{,}136.46 = 1.10$, a nonsignificant value for $n_1 = 24$ and $n_2 = 72$ degrees of freedom, since uniformity trial data were used. If significance of differences between variety means is indicated by the test of significance applied to the randomized-complete-block analysis, no further test of significance is necessary. Usually, when large numbers of varieties are used, it may be expected that significant differences between varieties will be found if the conditions of the test have been satisfactory.

The average yield of the varieties, calculated from Table 74, is affected by differences in productivity of the blocks. The necessary corrections are obtained by weighting c_x and c_y to give $c_{x'}$ and $c_{y'}$. These corrections are then added to the arithmetic averages to give the adjusted mean yields.

The weighting factor is $(w - w')/(w + w')$, where $w = 1/E$ and $w' = 3/(4B - E)$, E and B being, respectively, the error and block mean squares in Table 75. If B is less than or equal to E, no adjustments for blocks are necessary, and the averages in Table 77 are the correct variety means.

The general formulas for estimating w and w' are as follows:
Two replications:

E = intrablock error mean square

B = mean square for component b, based on $2(k - 1)$ degrees of freedom

$$w = \frac{1}{E}, \qquad \text{and} \qquad w' = \frac{1}{2B - E}$$

Four replications:

E = intrablock error mean square

B = average mean square of components a and b, based on $4(k - 1)$ degrees of freedom

$$w = \frac{1}{E} \qquad \text{and} \qquad w' = \frac{3}{4B - E}$$

Six replications:

E = intrablock error mean square

B = mean square for component a, based on $4(k - 1)$ degrees of freedom. Component b need not be used

$$w = \frac{1}{E} \qquad \text{and} \qquad w' = \frac{1}{B}$$

In this problem

$$w = \frac{1}{E} = \frac{1}{3818.89} = 0.00026186$$

and

$$w' = \frac{3}{4B - E} = \frac{3}{4(27{,}747.94) - 3818.89} = 0.00002799$$

The weighting factor is

$$\frac{w - w'}{w + w'} = \frac{0.00023387}{0.00028985} = 0.80687$$

The rkc_x and rkc_y values are multiplied by this weighting factor to secure the $c_{x'}$ and $c_{y'}$ corrections. Thus

$$c_{x'} = \frac{1}{rk}\left(\frac{w - w'}{w + w'}\right) rkc_x = \frac{1}{4 \times 5}(0.80687)rkc_x = 0.040344rkc_x$$

$$c_{y'} = \frac{1}{rk}\left(\frac{w - w'}{w + w'}\right) rkc_y = 0.040344rkc_y$$

In Table 77 are given the average yields of the varieties, obtained by dividing the totals in Table 74 by 4. The values of $c_{x'}$ and $c_{y'}$ are given at the side and bottom, respectively, of Table 77. The first $c_{x'}$ will be

$$(0.040344)(-405) = -16.34$$

The other values of $c_{x'}$ and $c_{y'}$ are calculated in a similar manner.

The adjusted variety means are secured by adding to each variety average in Table 77 the corrections in the same row and column. Thus, for variety 1, the adjusted (unconfounded) variety mean will be 587.50 − 16.34 + 14.32 = 585.5. Proceeding in a similar manner, the adjusted means of all varieties are calculated and entered in Table 78.

As an illustration, consider again the adjustment made for variety 1. It was shown previously that varieties, 1, 2, 3, 4, 5 yielded 6260 − 5855 = 405 g. more in the two blocks containing this group of varieties than the sum of the yields of the same varieties grown in different blocks (see Table 73). In like

manner, varieties 1, 6, 11, 16, 19 yielded 5465 − 5820 = −355 g. less in the two blocks containing them than the sum of the yields of the same varieties when each was grown in a different block.

TABLE 77.—AVERAGE YIELD OF VARIETIES AND c' VALUES

						$c_{x'}$
	1 587.50	2 572.50	3 586.25	4 660.00	5 622.50	−16.34
	6 552 50	7 590.00	8 720.00	9 696 25	10 552.50	−11.10
	11 580.00	12 548.75	13 617.50	14 621.25	15 601.25	17.95
	16 562.50	17 618.75	18 688.75	19 670.00	20 638.75	−43.37
	21 538.75	22 565.00	23 550.00	24 606.25	25 590 00	7 67
$c_{y'}$	14 32	31.06	2.02	−10.29	8.07	

TABLE 78.—ADJUSTED VARIETY MEANS

1 585.5	2 587.2	3 571.9	4 633.4	5 614.2
6 555.7	7 610.0	8 710.9	9 674.9	10 549.5
11 612.3	12 597.8	13 637.5	14 628.9	15 627.3
16 533.5	17 606.4	18 647.4	19 616.3	20 603.5
21 560.7	22 603.8	23 559.7	24 603.6	25 605.7

The mean of these differences is $\frac{(405) + (-355)}{20} = 2.50$. Subtracting 2.50 from the average yield of variety 1, 587.50 (see Table 77) would give 585.0 as the adjusted mean. This is the adjusted mean yield given by the original method of analysis developed by Yates (1936) and illustrated by Yates (1936) and

Goulden (1937, 1939). In the present analysis, the interblock information has been recovered, and the correction must be multiplied by the weighting factor $(w - w')/(w + w')$. Subtracting (2.50) (0.80687) from the average yield of 587.50 gives 585.5 as the adjusted mean. The method of computation used in the problem simplifies the calculation of these correction terms.

To calculate the standard error of the difference between variety means, the intrablock error mean square (Table 75) is an estimate of the uncontrolled error variance (s^2) of a single plot.

The standard error of the difference between the means of two varieties that have occurred in the same block, such as 1 and 2, 2 and 7, etc., is

$$\sqrt{\frac{2s^2}{rk}\left[\frac{2w}{w+w} + (k-1)\right]}$$

$$= \sqrt{\frac{(2)(3818.89)}{(4)(5)}\left[\frac{(2)(.00026186)}{(.00026186) + (.00002799)} + (5-1)\right]}$$

$$= \sqrt{2217.58} = 47.1$$

The standard error of the difference between the means of two varieties that did not occur in the same block, such as 1 and 7, is

$$\sqrt{\frac{2s^2}{rk}\left[\frac{4w}{w+w} + (k-2)\right]} = \sqrt{2525.71} = 50.3$$

The mean standard error of all comparisons is

$$\sqrt{\frac{2s^2}{r(k+1)}\left[\frac{4w}{w+w'} + (k-1)\right]} = \sqrt{2423.00} = 49.2$$

Usually, this latter standard error may be used for all comparisons of differences between variety means.

If the data had been analyzed as a randomized complete block (Table 76) the variance of the difference between two variety means would have been

$$\frac{2s^2}{r} = \frac{(2)(9136.46)}{4} = 4568.23$$

Assuming the precision obtainable in a randomized-complete-block design to be 100 per cent, the lattice design would be

4568.23/2423.00 = 189 per cent. This represents a gain of 89 per cent in precision through the use of incomplete blocks.

To make an exact test of significance appropriate to the analysis of a lattice experiment, it is necessary to correct the variety mean square so that this can be compared with intrablock error. This requires that the variety mean square be freed of block differences. To do so, we must first calculate the unadjusted sum of squares for component b of the blocks. This is calculated from the totals of the two sets of blocks given in Table 73. Numerically, this is

$$\frac{(6260)^2 + \cdots + (5605)^2 + (5466)^2 + \cdots + (5910)^2}{10} - \frac{(30{,}835)^2 + (29{,}715)^2}{50} = 222{,}136$$

for 8 degrees of freedom. This may be designated B_u. The adjusted sum of squares for component b (B_a) was given in Table 75 as 97,705. The adjusted sum of square for varieties (eliminating blocks) will be the sum of squares for varieties in Table 75, which is 240,663, minus $\left(\frac{w - w'}{w} B_u - \frac{w - w'}{w + w'} B_a\right)$. This becomes

$$240{,}663 - \left[\frac{(.00023387)}{.00026186}(222{,}136) - \frac{(.00023387)}{.00028985}(97{,}705)\right] = 121{,}106$$

Dividing this sum of squares by 24 gives 5046.08 as the adjusted mean square for varieties. The exact value of F is then 5046.08/3818.89 = 1.32.

Lattice experiments can be analyzed as randomized complete blocks, although when many treatments are included the error may be rather large. If it is found that B is equal to or less than E, there would be no advantage in adjusting the variety means. The unadjusted variety means should be used and the error obtained from a randomized-complete-block analysis. When a complete replication is lost the data may be analyzed as an ordinary randomized-block test. Methods of analysis appropriate for a lattice design may be used for the yield data,

the adjusted means being used, and other characters of less interest may be analyzed as ordinary randomized blocks, the unadjusted means being used.

TRIPLE LATTICE EXPERIMENTS

In triple lattice experiments,[1] the number of groups is three. The third group (Z) is added to the X and Y groups used in a simple lattice design. The number of replications must be a multiple of three. The number of varieties tested is the square of some number.

It is always possible to superimpose a Latin-square arrangement on a square of variety numbers. Using the five letters A, B, C, D, E, a Latin square of these letters is superimposed on the $k^2 = 25$ varieties, as given below:

1A	2E	3D	4C	5B
6B	7A	8E	9D	10C
11C	12B	13A	14E	15D
16D	17C	18B	19A	20E
21E	22D	23C	24B	25A

These 25 varieties may now be arranged in incomplete blocks of $k = 5$ varieties each. In the first group, designated X, the five rows of the square are arranged in random order, and the five varieties within each row (block) are randomized. The same procedure with the columns of the square produces the Y group. For the third, or Z group, the Latin letters are arranged in random order and the varieties with the same Latin letter randomized within each Latin-letter block. The blocks in group Z were made up of varieties with the Latin letters in the order A, E, C, B, D.

A random arrangement in three such groups is given in Table 79.

This random arrangement of "varieties" was superimposed on the uniformity trial data with rod rows of wheat given by Wiebe (1935). It was assumed that three row plots per variety were grown and only the central row harvested. The yields are given in grams per rod row. Three complete replications were used.

Assembling the yields for the X, Y, and Z groups according to rows, columns, and Latin letters gives Table 80. The block

[1] The triple lattice design has been called a two-dimensional pseudofactorial arrangement in three groups of sets and a two-dimensional quasi-factorial design in randomized blocks in three equal groups of sets.

totals are given also, as well as the total for each complete replication.

TABLE 79.—RANDOM ARRANGEMENT OF VARIETIES IN TRIPLE LATTICE EXPERIMENT

Replication 1 (Group X)

Block					
(1)	10	7	6	8	9
(2)	14	13	11	15	12
(3)	2	4	5	3	1
(4)	25	24	23	21	22
(5)	18	16	17	20	19

Replication 2 (Group Y)

Block					
(6)	15	5	10	20	25
(7)	16	6	21	11	1
(8)	2	17	7	22	12
(9)	23	3	13	18	8
(10)	24	4	14	19	9

Replication 3 (Group Z)

Block					
(11)	7	1	19	25	13
(12)	14	21	8	2	20
(13)	11	10	23	17	4
(14)	5	24	18	12	6
(15)	15	16	9	3	22

If six or nine replications were to be used, the X, Y, and Z groups would be repeated once or twice, respectively. In Table 81 is given the sum of the yields of each variety in a 5 by 5 table with appropriate row and column totals. To the

TABLE 80.—YIELDS OF VARIETIES IN GRAMS PER ROD ROW

Replication 1 (Group X)

Block						Block totals
(3)	1 635	2 525	3 555	4 650	5 635	3,000
(1)	6 495	7 730	8 810	9 775	10 710	3,520
(2)	11 630	12 600	13 645	14 635	15 645	3,155
(5)	16 735	17 690	18 840	19 855	20 805	3,925
(4)	21 620	22 795	23 590	24 660	25 615	3,280
						16,880

TABLE 80.—YIELDS OF VARIETIES IN GRAMS PER ROD ROW.—*(Continued)*

Replication 2 (Group *Y*)

Block						Block totals
(7)	1 530	6 490	11 595	16 495	21 540	2,650
(8)	2 610	7 660	12 620	17 695	22 570	3,155
(9)	3 705	8 850	13 675	18 685	23 640	3,555
(10)	4 840	9 905	14 785	19 860	24 875	4,265
(6)	5 670	10 455	15 655	20 665	25 615	3,060
						16,685

Replication 3 (Group *Z*)

Block						Block totals
(11)	1 580	7 675	13 545	19 470	25 570	2,840
(14)	6 700	12 620	18 575	24 570	5 505	2,970
(13)	11 515	17 450	23 550	4 495	10 550	2,560
(15)	16 640	22 700	3 635	9 645	15 640	3,260
(12)	21 445	2 515	8 465	14 445	20 455	2,325
						13,955

right of the table are given also the total yields of five varieties according to the Latin letters.

The analysis of variance may now be calculated. This procedure will follow closely the form given by Cox, Eckhardt, and Cochran (1940).

The correction term is $(47{,}520)^2 \div 75 = 30{,}108{,}672$. Total sum of squares is calculated from the sum of the squares of

the 75 individual plot yields minus the correction term. Thus, 31,090,500 − 30,108,672 = 981,828.

TABLE 81.—TOTAL YIELD OF VARIETIES

						Row totals	Latin letters	Totals
	1 1745	2 1650	3 1895	4 1985	5 1810	9,085	*A*	9,660
	6 1685	7 2065	8 2125	9 2325	10 1715	9,915	*B*	9,540
	11 1740	12 1840	13 1865	14 1865	15 1940	9,250	*C*	9,055
	16 1870	17 1835	18 2100	19 2185	20 1925	9,915	*D*	10,095
	21 1605	22 2065	23 1780	24 2105	25 1800	9,355	*E*	9,170
Column totals..	8645	9455	9765	10,465	9190	47,520		47,520

The sum of squares for replications is

$$\frac{(16{,}880)^2 + (16{,}685)^2 + (13{,}955)^2}{25} - 30{,}108{,}672 = 213{,}954$$

The sum of squares for varieties (ignoring blocks) is calculated from the variety totals in Table 81. This is

$$\frac{(1745)^2 + (1650)^2 + \cdots + (1800)^2}{3} - 30{,}108{,}672 = 260{,}561$$

In the triple lattice with three replications, there will be no component *a* for blocks, as described for four replications in the simple lattice design. Component *b* consists of three sets of values that may be used to give an estimate of block differences freed of varietal effects. The first row total in group *X*(3000) contains the yields of varieties 1, 2, 3, 4, 5. An unconfounded estimate of the sum of the yield of these five varieties can be obtained from the first column (Table 80) in group *Y*(3355) and for these same varieties in group *Z*(2730). An estimate of the block effect freed of varietal differences will be

given by

$$2(3000) - 3355 - 2730 = -85$$

This value can be calculated more conveniently by subtracting the total of the first row in Table 81 from three times the total of the first row in Table 80. Thus

$$3(3000) - 9085 = -85$$

These values are to be used in making the adjustments to the variety means. Since it will be easier to add than to subtract, in making the adjustments, the negative values are determined. These are designated as $2\,rkc_x$, $2\,rkc_y$, $2\,rkc_z$. They are calculated as follows:

$2\,rkc_x$ = row total of Table 81—3 (row total of group X, Table 80)

$2\,rkc_y$ = column total of Table 81—3 (row total of group Y, Table 80)

$2\,rkc_z$ = Latin-letter total of table 81—3 (row total of group Z, Table 80)

The values of $2\,rkc$ are calculated below:

$2\,rkc_x$		$2\,rkc_y$	
9085 − 3(3000) =	85	8,645 − 3(2650) =	695
9915 − 3(3520) =	− 645	9,455 − 3(3155) =	− 10
9250 − 3(3155) =	− 215	9,765 − 3(3555) =	− 900
9915 − 3(3925) =	−1860	10,465 − 3(4265) =	−2330
9355 − 3(3280) =	− 485	9,190 − 3(3060) =	10
	−3120		−2535

$2\,rkc_z$	
9,660 − 3(2840) =	1140
9,540 − 3(2970) =	630
9,055 − 3(2560) =	1375
10,095 − 3(3260) =	315
9,170 − 3(2325) =	2195
	5655

The sum of the $2\,rkc$ values must be 0.

$$(-3120) + (-2535) + (5655) = 0$$

The sum of squares of the deviations of the 2 *rkc* values within sets will be

$$\frac{(85)^2 + \cdots + (-485)^2 + (695)^2 + \cdots + (10)^2 + (1140)^2 + \cdots + (2195)^2}{30} - \frac{(-3120)^2 + (-2535)^2 + (5655)^2}{150} = 646{,}360 - 320{,}931 = 325{,}429$$

for 12 degrees of freedom. The divisors are 2 *rk* and 2 rk^2.

The results are now summarized in Table 82.

TABLE 82.—ANALYSIS OF VARIANCE OF TRIPLE LATTICE EXPERIMENT

Variation due to	Degrees of freedom	Sum of squares	Mean square
Replications	2	213,954	106,977.00
Component *b* for blocks (eliminating varieties)	12	325,429	27,119 08
Varieties (ignoring blocks)	24	260,561	10,856.71
Error (intrablock)	36	181,884	5,052.33
Total	74	981,828	

A test of significance of variety mean square cannot be made from the mean squares for varieties and error of the foregoing analysis, since variety mean square contains some block effects and an exact test requires additional computation and will be given later. An approximate test may be made in the form of a randomized-complete-block analysis by combining the degrees of freedom and sums of squares for blocks and error in Table 82 to produce Table 83.

TABLE 83.—ANALYSIS OF VARIANCE AS RANDOMIZED COMPLETE BLOCKS

Variation due to	Degrees of freedom	Sum of squares	Mean square	*F*
Replications	2	213,954	106,977.00	
Varieties	24	260,561	10,856 71	1.03
Error	48	507,313	10,569.02	
Total	74	981,828		

$F = 1.03$ is nonsignificant for $n_1 = 24$ and $n_2 = 48$ degrees of freedom, the varieties being hypothetical. If this test of significance showed a significant variety mean square, no further test would be necessary. If, however, the F value did not reach significance and block mean square in Table 82 were above error mean square, it would be well to make the exact test of significance.

To calculate the adjusted variety means the values of $2rkc_x$, $2rkc_y$, and $2rkc_z$ must be multiplied by a weighting factor to obtain the three sets of corrections $c_{x'}$, $c_{y'}$, and $c_{z'}$, respectively. The weighting factor is $\frac{2(w - w')}{2w + w'}$, where $w = 1/E$ and $w' = 2/(3B - E)$, E and B being the error and block mean squares, respectively, obtained from Table 82.

The general formulas for estimating w and w' are as follows:
Three replications:

E = intrablock error mean square

B = mean square for component b, based on $3(k - 1)$ degrees of freedom Component a does not exist

$$w = \frac{1}{E} \qquad \text{and} \qquad w' = \frac{2}{3B - E}$$

Six replications:

E = intrablock error mean square

B = average mean square for components a and b for $6(k - 1)$ degrees of freedom

$$w = \frac{1}{E} \qquad \text{and} \qquad w' = \frac{5}{6B - E}$$

Nine replications:

E = intrablock error mean square

B = mean square for component a for $6(k - 1)$ degrees of freedom. Component b need not be used

$$w = \frac{1}{E} \qquad \text{and} \qquad w' = \frac{1}{B}$$

If B is less than or equal to E, the randomized-complete-block analysis is to be used. The unweighted variety means are then tested with the error calculated from the randomized-complete-block analysis.

In this problem, the mean square for blocks (Table 82) is much greater than error mean square, and the weighting of the variety

means will lead to an increase in precision. Referring to the mean squares in Table 82,

$$w = \frac{1}{E} = \frac{1}{5052.33} = 0.00019793$$

$$w' = \frac{2}{3B - E} = \frac{2}{3(27,119.08) - 5,052.33} = 0.00002621$$

The weighting factor is then

$$\frac{2(w - w')}{2w + w'} = 0.81370$$

The weighted correction terms will be

$$c_{x'} = \frac{1}{2rk} \frac{2(w - w')}{2w + w'} (2rkc_x) = 0.027123(2rkc_x)$$

$$c_{y'} = \frac{1}{2rk} \frac{2(w - w')}{2w + w'} (2rkc_y) = 0.027123(2rkc_y)$$

$$c_{z'} = \frac{1}{2rk} \frac{2(w - w')}{2w + w'} (2rkc_z) = 0.027123(2rkc_z)$$

In Table 84 are given the average yields of the three replications of each variety, found by dividing the total yields in

TABLE 84.—AVERAGE YIELDS AND c' VALUES

						$c_{x'}$
	1A 581.67	2E 550.00	3D 631 67	4C 661.67	5B 603.33	2.31
	6B 561.67	7A 688.33	8E 708.33	9D 775.00	10C 571.67	−17.49
	11C 580 00	12B 613.33	13A 621.67	14E 621.67	15D 646.67	− 5.83
	16D 623.33	17C 611.67	18B 700.00	19A 728.33	20E 641.67	−50.45
	21E 535.00	22D 688.33	23C 593.33	24B 701.67	25A 600.00	−13.15
$c_{y'}$	18.85	−0.27	−24.41	−63.20	0.27	
$c_{z'}$	A 30.92	B 17.09	C 37.29	D 8.54	E 59.53	

Table 81 by 3. The weighted corrections $c_{x'}$, $c_{y'}$, and $c_{z'}$ are obtained by multiplying the values of $2rkc_x$, $2rkc_y$ and $2rkc_z$ by 0.027123. The first $c_{x'}$ is

$$(0.027123)(85) = 2.31$$

These are recorded for the proper row, column, or Latin letter in Table 84.

The adjusted mean yields are obtained by adding to the average yield of each variety the correction term in the same row, column and for the same Latin letter. For variety 1, the adjusted mean is 581.67 + 2.31 + 18.85 + 30.92 = 633.8. Proceeding in a similar manner for all varieties gives the adjusted means in Table 85.

TABLE 85.—ADJUSTED VARIETY MEANS

1 633.8	2 611.6	3 618.1	4 638.1	5 623.0
6 580.1	7 701.5	8 726.0	9 702.9	10 591 7
11 630.3	12 624.3	13 622.4	14 612.2	15 649 7
16 600.3	17 598.2	18 642.2	19 645.6	20 651 0
21 600.2	22 683.5	23 593 1	24 642.4	25 618 0

The mean of the 25 adjusted variety means will be the same as the mean of the 25 unadjusted means. Three times the total of Table 85 will be the grand total of Table 81.

Using $r = 3$ replications, $k = 5$ plots per block, and $s^2 = 5052.33$ (the error mean square in Table 82), the standard error of the difference between the adjusted mean yields of two varieties having occured in the same block will be

$$\sqrt{\frac{2s^2}{rk}\left\{\frac{6w}{2w + w'} + (k - 2)\right\}}$$

$$= \sqrt{\frac{2(5052.33)}{(3)(5)}\left\{\frac{6(.00019793)}{2(.00019793) + .00002621} + (5 - 2)\right\}}$$

$$= \sqrt{3916.36} = 62.6$$

The standard error of the difference between two varieties that did not occur in the same block is

$$\sqrt{\frac{2s^2}{rk}\left\{\frac{9w}{2w + w'} + (k - 3)\right\}} = \sqrt{4190.44} = 64.7$$

The mean standard error of the difference of all comparisons is

$$\sqrt{\frac{2s^2}{r(k + 1)}\left\{\frac{9w}{2w + w'} + (k - 2)\right\}} = \sqrt{4053.41} = 63.7$$

This latter standard error usually may be used for all comparisons without appreciable error.

To test the efficiency of the triple lattice design, we may divide the error variance of the difference between two variety means as calculated from the randomized-complete-block analysis by the error variance obtained through use of the triple-lattice arrangement.

The variance of the difference between two means by randomized-complete-block design would be $\frac{2s^2}{r} = \frac{2(10{,}569.02)}{3} = 7046.01$. Dividing 7046.01 by 4053.41 gives 174 per cent as the precision of the lattice design, when the ordinary randomized block is considered 100 per cent. Reducing the block size from 25 plots per complete block to 5 plots per incomplete block resulted in a gain in precision of 74 per cent.

The exact test of significance appropriate for the triple lattice design will now be illustrated. To make this test, it is necessary to calculate the variety mean square freed of block effects. The sum of squares for varieties (ignoring blocks), given in Table 82, must be diminished by

$$\frac{2(w - w')}{2w} B_u - \frac{2(w - w')}{2w + w'} B_a$$

where B_u and B_a are the unadjusted and adjusted sums of squares, respectively, for component b of the blocks.

B_u will be the sum of squares between blocks, within sets, calculated from the block totals in Table 80. Thus

$$\frac{(3000)^2 + \cdots + (3280)^2 + (2650)^2 + \cdots + (3060)^2 + (2840)^2 + \cdots + (2325)^2}{5} - \frac{(16{,}880)^2 + (16{,}685)^2 + (13{,}955)^2}{25} = 507{,}404$$

B_a was given in Table 82 as 325,429. Then

$$\frac{2(w - w')}{2w} B_u - \frac{2(w - w')}{2w + w'} B_a = (0.867579)(507,404) - (0.813704)(325,429) = 175,410$$

Subtracting this quantity from the unadjusted sum of squares for varieties gives 260,561 − 175,410 = 85,151 as the adjusted sum of squares for varieties. Dividing by 24 degrees of freedom gives 3547.96 as the corrected mean square. Since this is less than the mean square for error (5052.33, Table 82), F will be less than 1. A lack of significance is clearly indicated.

ANALYSIS OF DATA EXPRESSED AS PERCENTAGES

In the analysis of data expressed in the form of a binomial, such as is frequently encountered in studies of the proportion, or percentage, of plants diseased, the standard error of the proportion will be given by $\sqrt{pq/N}$, where p is the proportion diseased, $q = (1 - p)$ is the proportion disease free, and N is the total number of plants in the sample. If, for example, one-fourth of the plants in a sample of 100 were diseased, the standard error of $p = 0.25$ would be $\sqrt{\frac{(0.25)(0.75)}{100}} = \sqrt{0.001875} = 0.043$. If expressed as $p = 25$ per cent, the standard error would be 4.3 per cent.

The standard error of a proportion, or percentage, is clearly dependent on the value of p as well as N, being a maximum when $p = 0.50$ and reducing to zero as p becomes 0 or 1.00. Since a basic assumption in the use of a generalized error from an analysis of variance is that the errors of the separate treatments must be independent of the means, data expressed as percentages frequently need to be transformed before being analyzed by means of an analysis of variance. The transformation would need to be one for which the variance of each treatment is equalized and dependent on N alone. Bliss (1937) suggested an angular transformation in which p is replaced by $\sin^2 \theta$. Tables for making such transformations have been given by Bliss (1937, 1938), Fisher and Yates (1938), and Snedecor (1940) and are reproduced as Appendix Table VI. A discussion of some of the difficulties in the analysis of data of this type has been given by Cochran (1938).

The use of such transformations will be illustrated with data taken from a paper by Salmon (1938). Clark and Leonard (1939) have given a full analysis of these data. In Table 86 is given the percentage of bunt on each of 5 varieties of wheat inoculated with 10 different collections of the organism. Two replications were used. The percentages were based on counts of 200 to 400 heads per plot.

TABLE 86.—PERCENTAGE INFECTION IN DIFFERENT VARIETIES OF WHEAT WITH 10 COLLECTIONS OF BUNT, IN EACH OF TWO REPLICATIONS (FROM SALMON)

Bunt collection number	Hybrid 128		Minturki		Turkey		Albit		Ridit		Total
	I	II	I	II	I	II	I	II	I	II	
2	76	95	91	84	89	84	92	91	9	2	713
3	95	93	88	75	3	6	94	90	6	1	551
4	91	92	92	83	82	87	14	5	4	3	553
5	84	90	61	81	8	2	4	3	4	4	341
7	98	98	56	44	14	9	0	1	2	2	324
10	94	83	71	64	6	1	92	80	4	4	499
11	83	78	71	70	4	1	2	4	7	6	326
51	94	96	45	40	28	22	1	3	5	5	339
157	75	86	75	85	52	92	89	85	1	1	641
189	87	95	81	80	80	92	92	95	5	6	713
Total	877	906	731	706	366	396	480	457	47	34	5000

These data were transformed into the form $p = \sin^2 \theta$ by means of Appendix Table VI. The transformed data are given in Table 87.

These transformed data in Table 87 may be subjected to an analysis of variance. The correction term will be $\frac{(4361.7)^2}{100} = 190{,}244.27$. The total sum of squares will be $269{,}490.93 - 190{,}244.27 = 79{,}246.66$. The sum of squares for replication is obtained by adding the totals for replicates 1 and 2, respectively, for all five varieties to give 2188.4 and 2173.3. The sum of squares for replication is $\frac{(2188.4)^2 + (2173.3)^2}{50}$ minus the correction term or 2.28.

In Table 88 are added the values of the transformations for the two replications to give the totals for both.

TABLE 87.—PERCENTAGE DATA FROM TABLE 86 TRANSFORMED TO DEGREES BY MEANS OF THE TRANSFORMATION $p = \sin^2 \theta$

Bunt collection number	Hybrid 128		Minturki		Turkey		Albit		Ridit		Total
	I	II	I	II	I	II	I	II	I	II	
2	60 7	77 1	72 5	66.4	70.6	66 4	73 6	72.5	17 5	8 1	585.4
3	77.1	74 7	69 7	60 0	10 0	14 2	75 8	71 6	14 2	5 7	473 0
4	72.5	73.6	73 6	65.7	64 9	68 9	22 0	12.9	11.5	10.0	475 6
5	66.4	71.6	51 4	64.2	16.4	8.1	11.5	10.0	11.5	11 5	322.6
7	81.9	81 9	48.5	41.6	22 0	17 5	0.0	5 7	8.1	8 1	315 3
10	75.8	65.7	57.4	53.1	14.2	5.7	73.6	63.4	11.5	11.5	431.9
11	65.7	62.0	57 4	56.8	11.5	5 7	8.1	11.5	15.3	14 2	308 2
51	75 8	78.5	42 1	39 2	32 0	28 0	5.7	10 0	12.9	12.9	337.1
157	60.0	68 0	60.0	67 2	46.2	73.6	70.6	67.2	5.7	5 7	524 2
189	68 9	77.1	64 2	63.4	63.4	73 6	73 6	77.1	12 9	14.2	588.4
Total	704 8	730 2	596.8	577.6	351.2	361 7	414 5	401 9	121.1	101 9	4361 7

TABLE 88.—TOTALS OF TWO REPLICATIONS FOR EACH VARIETY AND BUNT COLLECTION

Bunt collection number	Hybrid 128	Minturki	Turkey	Albit	Ridit	Total
2	137 8	138 9	137 0	146 1	25.6	585.4
3	151.8	129 7	24 2	147 4	19 9	473.0
4	146 1	139 3	133 8	34 9	21 5	475.6
5	138.0	115.6	24 5	21 5	23.0	322.6
7	163.8	90 1	39 5	5 7	16 2	315.3
10	141.5	110 5	19 9	137.0	23.0	431.9
11	127.7	114.2	17.2	19 6	29.5	308.2
51	154 3	81 3	60 0	15 7	25 8	337.1
157	128 0	127.2	119 8	137.8	11.4	524.2
189	146.0	127 6	137 0	150 7	27.1	588.4
Total...	1435.0	1174.4	712.9	816.4	223.0	4361.7

The sum of squares for bunt collections will be

$$\frac{(585.4)^2 + \cdots + (588.4)^2}{10} - 190{,}244.27 = 10{,}982.11$$

The sum of squares for varieties will be

$$\frac{(1435.0)^2 + \cdots + (223.0)^2}{20} - 190{,}244.27 = 42{,}900.97$$

The sum of squares for varieties inoculated with the individual bunt collections is calculated from the 50 figures within Table 88. Thus $\frac{(137.8)^2 + (138.9)^2 + \cdots + (27.1)^2}{2} - 190{,}244.27 =$ 77,937.02. The sum of squares for interaction of varieties × collections will be

$$77{,}937.02 - 10{,}982.11 - 42{,}900.97 = 24{,}053.94$$

The analysis of variance is given in Table 89.

TABLE 89.—ANALYSIS OF VARIANCE OF TRANSFORMED DATA

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Blocks	1	2 28	2 28	
Varieties	4	42,900.97	10,725 24	402 00*
Collections	9	10,982.11	1,220 23	45 74*
Varieties × collection	36	24,053 94	668.17	25.04*
Error	49	1,307.36	26 68	
Total	99	79,246 66		

* Exceeds the 1 per cent point.

The varieties gave highly significant differences in their average reaction to all collections of bunt. The bunt collections differed significantly in their ability to produce the disease, as an average of all varieties. Furthermore, the interaction of varieties × collections was highly significant, indicating clearly that these collections produced differential responses on the different varieties.

The standard error of the difference between the means of varieties for all bunt collections would be $\sqrt{(2 \times 26.68)/20} = 1.63$. The totals in table could be compared also. The standard error of the difference between means is $\sqrt{2s^2/N}$, and the standard error of the difference between totals is $\sqrt{2s^2N}$. The standard error of the difference between two variety totals would be $\sqrt{2 \times 26.68 \times 20} = 32.67$.

The standard error for determining significant interactions of the totals for two replications would be $\sqrt{2 \times 2 \times s^2 \times N} =$

$\sqrt{2 \times 2 \times 26.68 \times 2} = 12.65$. The difference between the differences in reaction of Turkey and Albit to collections 3 and 4 is seen to be $(24.2 - 133.8) - (147.4 - 34.9) = -221.1$. Since this difference is 15.1 times its standard error, it is clear that these two varieties reacted in a differential manner to collections 3 and 4. Other comparisons could be made in a similar manner.

If the range in the percentages is between about 25 and 75, no transformation probably would need to be made. In this range, the errors of the separate varieties would be sufficiently similar so that a transformation would be unnecessary. If, however, the range in percentages goes below 25 or above 75, a transformation probably would be worth while. Such would be true particularly if some of the percentages were very low or very high.

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GLOSSARY

Allele, allel, allelomorph: adjective forms: allelic, allelomorphic.—One of a pair, or of a series of factors, that occur at similar loci of homologous chromosomes and for this reason are inherited in alternative pairs. One alternative form of a gene.

Aleurone.—The protein grains found in the endosperm of ripe seeds.

Aleurone layer.—In wheat and maize, the outer differentiated layer of cells of the endosperm; named thus because these cells are filled with aleurone grains.

Allopolyploid.—A polyploid having chromosome sets from different sources, such as different species. A polyploid containing genetically different chromosome sets; for example, from two or more species.

Amphidiploid.—A plant possessing the sum of the somatic chromosome numbers of two species.

Andromonoecious.—A plant bearing bisexual or complete flowers instead of strictly pistillate ones in addition to staminate flowers.

Aneuploid.—An organism or cell having a chromosome number other than an exact multiple of the monoploid or basic number. Hyperploid = higher. Hypoploid = lower.

Anthesis.—The period or act of flowering.

Apogamy.—The development of a sporophyte from some other cell or cells of the gametophyte (embryo sac) instead of from a gamete (egg).

Apomixis.—The development of an individual from an unfertilized egg without sexual fusion, whether the egg be normally haploid or abnormally diploid through failure of reduction division.

Autogamous.—Self-fertilizing.

Autopolyploid.—A polyploid arising through the multiplication of the complete genom complement of a species; *e.g.*, an autotetraploid has four identical sets of chromosomes.

Awn.—A bristle-shaped elongated appendage or extension to a glume, akene, anther, etc.

Backcross.—The cross of a hybrid to one of the parental types. The offspring of such a cross is referred to as the backcross generation.

Backcross method of breeding.—A system of breeding carried out by several generations of backcrossing and subsequent selection. The characters of the recurrent parent are retained for the most part, and a few characters from the nonrecurrent parent are added.

Biometry.—The application of statistical methods to the study of biological problems.

Biotype.—A population of individuals with identical genetic constitution. A biotype may be homozygous or heterozygous.

Bivalent.—A pair of synapsed or associated homologous chromosomes.

Bud-sport.—A branch, flower, or fruit that differs genetically from the remainder of the plant.

Bulk method of breeding.—The growing of segregating generations of a hybrid of self-pollinated crops in a bulk plot, with or without mass selection, followed by individual plant selection in F_6 or later generations.

Caryopsis.—A one-seeded dry fruit with the thin pericarp adherent to the seed, as in most grasses.

Chaff.—The floral parts of cereals, generally separated from the grain in threshing or winnowing.

Character.—One of the many details of structure, form, substance, or function that make up an individual organism. The Mendelian characters of genetics represent the end products of development, during which the entire complex of genes interacts within itself and with the environment.

Chimera.—A mixture of tissues of genetically different constitution in the same part of an organism. It may result from mutation, irregular mitosis, somatic crossing over, or artificial fusion (grafting). There are two main types, periclinial with parallel layers of genetically different tissues and sectorial.

Chi-square (χ^2) test.—A statistical comparison of observed with theoretical ratios.

Goodness of fit.—Comparison of observed Mendelian ratio with a theoretical.

Independence.—A test for association between two series of variables.

Chromatids.—Half chromosomes, resulting from longitudinal division, that later became daughter chromosomes.

Chromosomes.—Microscopically small, dark-staining bodies visible in the nucleus of the cell at the time of nuclear division. The number in any species is usually constant. They carry the genes, arranged in linear order.

Class.—A group that includes variates of similar magnitude.

Clon.—All the individuals derived by vegetative propagation from a single original individual.

Coefficient of variability.—A measure of variability expressed in percentage.

Combining ability.—The relative ability of a biotype to transmit desirable performance to its crosses.

Complementary genes.—Genes that interact to produce a new character.

Convergent improvement.—A system of double backcrossing for the purpose of improving each of two inbred lines without greatly modifying the yield of their F_1 cross.

Correlation coefficient.—A statistical measure of relationship between two or more series of variables.

Simple.—The total correlation between two series of variables.

Partial.—The correlation between two series of variables independent of the accompanying variation due to other variables.

Multiple.—A coefficient that measures the degree to which the dependent variable is influenced by a series of other factors studied.

Coupling.—The condition in linked inheritance in which an individual heterozygous for two pairs of factors received the two dominant members from one parent and the two recessives from the other parent; *e.g.*, $AABB \times aabb$.

Crossing over.—The exchange of corresponding segments between the chromatids of paired (homologous) chromosomes. It is a process inferred genetically from new associations of linked factors and inferred cytologically from new associations of parts of chromosomes, both of which may be observed in heterozygotes. It results in an exchange of factors and therefore in combinations of factors differing from those that came in with the parents. The term genetic crossover may be applied to these new gene combinations.

Cross-pollination.—The pollination of a plant by pollen of a different plant.

Deficiency.—The absence, "deletion," or inactivation of a segment of a chromosome.

Deletion.—The absence of a segment of a chromosome involving one or more genes.

Detassel.—To remove the tassel, as in maize.

Dioecious.—Having male and female flowers on different plants.

Diploid.—An organism with two sets of chromosomes.

Disease garden.—A special nursery for the study of reaction to specific pathogens.

Dominant.—A term applied to one member of an allelic pair of characters that has the quality of manifesting itself wholly or largely to the exclusion of the other member.

Double cross.—A term used particularly in corn, where four inbred lines are used as parents. The double cross is the F_1 cross between two single crosses.

Duplicate genes.—Two separately inherited factors, either alone or together, giving similar effects.

Duplication.—The occurrence of a segment more than once in the same chromosome or genom.

Ear.—A large, dense, or heavy spike or spike-like inflorescence, as the ear of maize. Popularly applied also to the spike-like panicle of such grasses as wheat, barley, timothy, and rye.

Emasculation.—The act of removing the anthers from a flower.

Endosperm.—The nutritive tissue formed within the embryo sac in seed plants. It commonly arises following the fertilization of the two primary endosperm nuclei of the embryo sac by one of the two male sperms. In a diploid organism, the endosperm is triploid.

Epistasis.—The suppression of a character dependent upon the action of a gene or genes by a gene or genes not allelic to those suppressed. Those characters suppressed are said to be hypostatic. Distinguished from dominance that refers to the members of one allelic pair.

Euploid.—An organism or cell having a chromosome number which is an exact multiple of the monoploid or haploid number. Terms used for a euploid series are haploid, diploid, triploid, tetraploid, etc.

F_1.—The first filial generation. The first generation of a given mating.

F_2.—The second filial generation, produced by crossing *inter se* or by self-pollinating the F_1.

Factor.—The same as gene.

Fatuoids.—Mutants occurring in cultures of cultivated oats and possessing characters of *Avena fatua*, wild oats.

Fertility.—The ability to produce viable offspring.

Fertilization.—The fusion of a male gamete (sperm) with a female gamete (egg) and of their nuclei, without which their later development is usually impossible.

Floret.—A small flower, especially one of an inflorescence, as in grasses and Compositae.

Foundation stock seed.—Seed that has descended from a selection of recorded origin, under the direct control of the original breeder, a delegated representative, or of a state or federal experiment station.

Gamete.—A mature male or female reproductive cell (sperm or egg).

Gene.—The hypothetical unit of inheritance located in the chromosome, which by interaction with the other genes and the environment controls the development of a character. Genes are believed to be arranged linearly in the chromosomes.

Genom.—A complete set of chromosomes (hence of genes), inherited as a unit from one parent.

Genotype.—The fundamental hereditary constitution of an organism.

Glabrous.—Smooth, without hairs.

Glume.—One of the two empty chaffy bracts at the base of each spikelet in grasses.

Grain.—Cereal seeds in bulk. Seed-like fruit of any cereal grass.

Haploid.—An organism or cell having only one complete set of chromosomes.

Head.—A dense, short cluster of sessile or nearly sessile flowers on a very short axis or receptacle, as in red clover or sunflower.

Heteroploid.—An organism characterized by a chromosome number other than the true euploid number.

Heterosis.—Hybrid vigor.

Heterozygous.—The condition in which the homologous chromosomes of an individual possess different genes of the same allelic series.

Homologous.—Chromosomes occur in somatic cells in pairs that are similar in size, shape, and supposedly in function, one being derived from the male and one from the female parent. The two members of such a pair are spoken of as homologous chromosomes.

Homozygous.—Possessing identical genes with respect to any given pair or series of alleles.

Hull.—The term applied to include the lemma and palea when they remain attached to the caryopsis after threshing.

Hybrid vigor.—The phenomenon in which the cross of two stocks produces hybrids that show increased vigor.

Hybrids.—The progeny of a cross-fertilization of parents belonging to different genotypes.

Hypostasis.—*See* Epistasis.

Inbred Line.—A relatively homozygous line produced by inbreeding and selection.

Inbred-variety Cross.—The F_1 cross of an inbred line with a variety.

Inflorescence.—The flowering part of a plant.

Interchange.—An exchange of segments of nonhomologous chromosomes.

Interference.—The property by which the occurrence of one crossover reduces the chance of occurrence of another in its neighborhood.

Inversion.—A rearrangement of a group of genes in a chromosome in such a way that their order in the chromosome is reversed.

Keel.—A central ridge resembling the keel of a boat, as in the glumes of some grasses, etc.; also, the inferior petal in the legume flowers.

Kernel.—The inner portion of a seed within the integuments. Also the whole grain of a cereal.

Latin square.—An experimental design for comparing treatments where the number of replications is the same as the number of treatments and each treatment occurs only once in each row and column. Especially adapted for accurate comparisons with a small number of treatments.

Lattice designs.—Designs developed for testing a large number of treatments, in which the number of blocks exceeds the number of complete replications.

Lethal gene.—A gene that renders inviable an organism or a cell possessing it.

Linkage.—Association of characters in inheritance, due to the fact that the genes determining them are physically located in the same chromosomes. Such a group of linked genes is called a linkage group.

Lodicule.—A minute scale at the base of the ovary opposite the palea in grasses, usually two in number, probably representing the reduced perianth.

Mass selection.—Selection for some desired character, or characters, where progeny of the plants or heads selected are grown in bulk.

Mature-plant resistance.—A term applied particularly to resistance to stem rust in the stages from heading to maturity where this resistance is not correlated with seedling reaction.

Mean.—The arithmetic average.

Megaspore (macrospore).—A spore having the property of giving rise to a gametophyte (embryo sac) bearing only a female gamete. One of the four cells produced by two meiotic divisions of the megaspore-mother-cell (megasporocyte).

Meiosis.—The process by which the chromatin material becomes reduced qualitatively and quantitatively to half the somatic number. It is completed in the two divisions, meiotic mitoses, which precede the formation of gametes in animals, or of spores in plants.

Microspore.—One of the four cells produced by the two meiotic divisions (mitoses) of the microspore-mother-cell (microsporocyte). A spore having the property of giving rise to a gametophyte bearing only male gametes.

Mitosis.—The process by which the nucleus is divided into two daughter nuclei.

Somatic mitosis.—The process by which the daughter nuclei are identical, quantitatively and qualitatively.

Meiotic mitoses.—Two nuclear divisions that result in spores in higher plants and in gametes in animals. Both divisions are necessary to complete reduction.

Mode.—The class of greatest frequency in a frequency distribution.

Modifier or modifying Gene.—A gene that affects the expression of another nonallelic gene.

Monoecious.—With separate male and female flowers on the same plant.

Multiple alleles.—A series of alleles in similar loci of homologous chromosomes of related races.

Multiple cross.—A cross between more than two parental lines of different origin.

Multiple-factor hypothesis.—The type of inheritance in which a character is dependent on many different genes or factors.

Mutation.—A sudden variation that is inherited. The term is used loosely to include "point mutations" of a single gene and chromosomal changes.

Nonrecurrent parent.—Used in backcrosses to refer to the original parent not used in backcross generations.

Ovary.—The swollen part of the pistil that contains the ovules.

Ovule.—The macrosporangium of flowering plants, consisting of the nucellus plus the integuments.

P_1, P_2, etc.—The first, second, etc., parental generation of a parent.

Palea.—The upper of the two bracts immediately enclosing each floret in grasses.

Panicle.—A compound inflorescence with pediceled flowers, usually loose and irregular, as in oats, rice, proso, etc.

Parthenogenesis.—The development of a new individual from a germ cell without fertilization.

Pedicel.—A stalk on which an individual blossom is borne.

Pedigree method of breeding.—A system of individual plant selection during the segregating generations of a cross where the progeny plants usually are separately spaced and the pedigree of particular selections is known.

Peduncle.—The primary stalk supporting either an inflorescence or a solitary flower. In grasses, the uppermost internode of the culm.

Pericarp.—The mature or ripened ovary wall around the ovule.

Phenotype.—The observed character of an individual without reference to its genetic nature. Individuals of the same phenotype look alike but may not breed alike.

Physiologic races.—Biotypes or groups of biotypes within species that behave more or less consistently in pathogenicity on certain differential varieties or host plants. Physiologic races sometimes are differentiated on the basis of cultural or physiochemical characters.

Physiological resistance.—A type of resistance due to physiological or protoplasmic incompatibility between the host plant and the pathogen.

Pistil.—That part of the flower consisting of ovary plus style and stigma.

Polyploid.—An organism with more than two sets of a basic or monoploid number of chromosomes, *e.g.*, triploid, tetraploid, pentaploid, hexaploid, heptaploid, octoploid, etc.

Proterandry.—The maturing and functioning of stamens before pistils in hermaphroditic flowers or in different flowers of the same plant in a monoecious species.

Proterogyny.—The reverse of proterandry.

Pubescent.—Hairy, in a general sense; in special use, covered with short soft hairs.

Pure line.—A strain of organisms that is comparatively pure genetically (homozygous) because of continued inbreeding or through other means.

Quadrivalent.—Association of homologous chromosomes in groups of four.

Qualitative characters.—Characters that are qualitatively different, so that separation is relatively easy.

Quantitative characters.—Characters that show a continuous range in variability, making separation into distinct classes difficult.

Randomized blocks.—An experimental design in which the treatments are arranged in random order within the blocks or replicates.

Recessive.—A term applied to one member of an allelic pair lacking the ability to manifest itself wholly or in part when the other or dominant member is present.

Recombination.—The observed new combinations of characters different from those combinations exhibited by the parents. Percentage of recombination equals percentage of crossing over only when the genes are relatively close together. Cytological crossing over refers to the process; recombination or genetic crossing over refers to the observed genetic result.

Reciprocal crosses.—Crosses where the parental plants or lines are used as both male and female.

Recurrent parent.—Used in backcrosses to refer to the parent to which the first cross and backcrossed plants are crossed.

Reduction division; heterotypic division.—Terms formerly applied to the one of the meiotic mitoses at which a particular author thought reduction and segregation occurred.

Registered seed.—Seed of a variety or strain that is the multiplied progeny of foundation stock seed and traces directly to it and that complies with certain standards of purity and quality.

Regression coefficient.—A coefficient that gives the rate of change in one variable (dependent variable) per unit rate of change in another (independent variable).

Replication.—Repetition of treatments in experiments.

Repulsion.—The condition in linked inheritance in which an individual heterozygous for two pairs of linked factors received the dominant member of one pair and the recessive member of the other pair from one parent and the reverse condition from the other parent; *e.g.*, $AAbb \times aaBB$.

Rod row.—A type of field plot approximately 1 rod long. Used particularly with small grains where the seed is sown without definite spacing.

Roguing.—The act of removing undesirable individuals from a varietal mixture in the field by hand selection.

Seed.—The mature ovule, consisting of the kernel and its integuments. Also used for the seedlike fruits of cereals.

Segregation.—The separation of the paternal from maternal chromosomes at meiosis and the consequent separation of differences as observed genetically in the offspring.

Self-fertilization.—The union of the egg cell of one individual with a sperm cell of the same individual.

Self-incompatibility.—Some physiological hindrance to self-fertilization.

Sibmating.—Crossing of siblings, two or more individuals of the same parentage (brother-sister mating).

Single cross.—A cross between two inbred lines.

Somatic.—Referring to body tissues; having two sets of chromosomes, one set normally coming from the female parent and one from the male, as contrasted with germinal tissue that will give rise to germ cells.

Somatoplastic sterility.—The collapse of fertilized ovules during the early developmental stages.

Species.—A group of individuals so much alike that it may reasonably be assumed that they have arisen from a common ancestor.

Speltoid.—Mutants occurring in cultures of common wheat, *Triticum vulgare*, and possessing characters of *T. spelta*, spelt wheat.

Spike.—A simple inflorescence with the flowers sessile or nearly so on a more or less elongated common axis or rachis.

Spikelet.—A small or secondary spike, especially in the inflorescence of grasses.

Standard deviation.—A measure of variability in terms of the units of measurement. Frequently refers to the infinite population.

Standard error.—Similar to standard deviation, except that it is calculated from a sample.

Sterility.—Inability to produce viable offspring.

Strain.—A group within a variety that constantly differs in genetic factors or a single genetic-factor difference from other strains of the same variety.

Strain building.—The improvement of cross-pollinated plants by any one of several methods of selection.

Synapsis.—The conjugation of homologous chromosomes.

Synthetic variety.—A term used particularly with cross-pollinated plants to refer to a variety produced by the combination of selected lines or plants and subsequent normal pollination.

***t* test.**—A method for testing the significance of a difference.

Three-way cross.—A cross between a single cross and an inbred line.

Top-cross.—*See* Inbred-variety cross.

Transgressive segregation.—The appearance in the F_2(or later) generations of individuals showing a more extreme development of a character than either parent. Assumed to be due to cumulative and complementary effects of genes contributed by the parents of the original hybrid. Adequate testing of variation in the parents is required to establish its occurrence.

Translocation.—The change in position of a segment of a chromosome to another part of the same chromosome or of a different chromosome.

Triploid.—An organism whose cells contain three haploid (monoploid) sets of chromosomes.

Trivalent.—An association of three homologous chromosomes at meiosis.

Unit character.—Term used for a character believed to be determined by the alleles at a single-gene locus. The term is now largely abandoned.

Univalent.—A chromosome unpaired at meiosis.

Variance.—The square of the standard deviation or standard error.

Xenia.—The immediate effect of pollen on the endosperm, due to the phenomenon of double fertilization in the seed plants.

Zygote.—The cell produced by the union of two cells (gametes) in reproduction; also, the individual developing from such a cell.

APPENDIX

TABLE I.—TABLE OF t*

Degrees of freedom	Probability (P)		Degrees of freedom	Probability (P)	
	.05	.01		.05	.01
1	12 71	63.66	26	2 06	2 78
2	4 30	9 92	27	2 05	2 77
3	3 18	5 84	28	2 05	2.76
4	2 78	4 60	29	2 04	2.76
5	2 57	4 03	30	2 04	2.75
6	2 45	3 71	35	2 03	2.72
7	2 36	3 50	40	2.02	2.70
8	2 31	3 36	45	2 01	2.69
9	2 26	3 25	50	2 01	2 68
10	2 23	3 17	60	2 00	2.66
11	2 20	3 11	70	1 99	2 65
12	2 18	3 06	80	1.99	2.64
13	2.16	3 01	90	1 99	2 63
14	2 14	2 98	100	1.98	2.63
15	2 13	2 95	125	1 98	2 62
16	2 12	2 92	150	1 98	2 61
17	2.11	2.90	200	1 97	2.60
18	2 10	2 88	300	1 97	2.59
19	2 09	2 86	400	1 97	2 59
20	2 09	2 84	500	1 96	2.59
21	2 08	2 83	1000	1 96	2.58
22	2 07	2 82	∞	1.96	2.58
23	2 07	2 81			
24	2 06	2 80			
25	2 06	2 79			

* Abridged from Table IV of Fisher's "Statistical Methods for Research Workers," Oliver & Boyd, Edinburgh, and from Table 16 of Wallace and Snedecor's "Correlation and Machine Calculation," by kind permission of the authors and publishers.

TABLE II.—POINTS FOR THE DISTRIBUTION OF F*

Values for 5 per cent in lightface type. Values for 1 per cent in boldface type

n_2	n_1 degrees of freedom (for greater mean square)																						
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
1	161	200	216	225	230	234	237	239	241	242	243	244	245	246	248	249	250	251	252	253	253	254	254	254
	4,052	**4,999**	**5,403**	**5,625**	**5,764**	**5,859**	**5,928**	**5,981**	**6,022**	**6,056**	**6,082**	**6,106**	**6,142**	**6,169**	**6,208**	**6,234**	**6,258**	**6,286**	**6,302**	**6,323**	**6,334**	**6,352**	**6,361**	**6,366**
2	18.51	19.00	19 16	19 25	19.30	19.33	19.36	19.37	19.38	19 39	19.40	19.41	19.42	19.43	19.44	19.45	19 46	19.47	19 47	19.48	19.49	19.49	19.50	19 50
	98.49	**99.01**	**99.17**	**99.25**	**99.30**	**99.33**	**99.34**	**99.36**	**99.38**	**99.40**	**99.41**	**99.42**	**99.43**	**99.44**	**99.45**	**99.46**	**99 47**	**99.48**	**99.48**	**99.49**	**99.49**	**99.49**	**99.50**	**99.50**
3	10.13	9.55	9.28	9.12	9.01	8.94	8.88	8.84	8.81	8.78	8.76	8 74	8.71	8.69	8.66	8.64	8.62	8.60	8.58	8.57	8.56	8.54	8.54	8.53
	34 12	**30.81**	**29.46**	**28.71**	**28.24**	**27.91**	**27.67**	**27.49**	**27.34**	**27.23**	**27.13**	**27.05**	**26.92**	**26.83**	**26.69**	**26.60**	**26.50**	**26.41**	**26.35**	**26.27**	**26.23**	**26.18**	**26.14**	**26.12**
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6 00	5.96	5.93	5 91	5 87	5.84	5.80	5.77	5.74	5 71	5.70	5.68	5.66	5.65	5.64	5.63
	21.20	**18.00**	**16.69**	**15.98**	**15.52**	**15.21**	**14.98**	**14.80**	**14.66**	**14.54**	**14.45**	**14.37**	**14.24**	**14.15**	**14.02**	**13.93**	**13.83**	**13.74**	**13.69**	**13.61**	**13.57**	**13.52**	**13.48**	**13.46**
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.78	4.74	4.70	4.68	4 64	4 60	4 56	4.53	4.50	4.46	4 44	4.42	4.40	4.38	4.37	4.36
	16.26	**13.27**	**12.06**	**11.39**	**10.97**	**10.67**	**10.45**	**10.27**	**10.15**	**10.05**	**9.96**	**9.89**	**9.77**	**9.68**	**9.55**	**9.47**	**9.38**	**9.29**	**9.24**	**9.17**	**9.13**	**9.07**	**9.04**	**9.02**
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.03	4 00	3.96	3.92	3.87	3.84	3 81	3.77	3.75	3.72	3.71	3.69	3.68	3.67
	13.74	**10.92**	**9.78**	**9.15**	**8.75**	**8.47**	**8.26**	**8.10**	**7.98**	**7.87**	**7.79**	**7.72**	**7.60**	**7.52**	**7.39**	**7.31**	**7.23**	**7.14**	**7.09**	**7.02**	**6.99**	**6.94**	**6.90**	**6.88**
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.63	3 60	3.57	3.52	3.49	3.44	3.41	3.38	3.34	3.32	3.29	3.28	3.25	3.24	3.23
	12.25	**9.55**	**8.45**	**7.85**	**7.46**	**7.19**	**7.00**	**6.84**	**6.71**	**6.62**	**6.54**	**6.47**	**6.35**	**6.27**	**6.15**	**6.07**	**5.98**	**5.90**	**5.85**	**5.78**	**5.75**	**5.70**	**5.67**	**5.65**
8	5.32	4.46	4.07	3.84	3.69	3.58	3 50	3.44	3.39	3.34	3.31	3.28	3.23	3.20	3.15	3.12	3.08	3.05	3.03	3.00	2.98	2.96	2.94	2.93
	11.26	**8.65**	**7.59**	**7.01**	**6.63**	**6.37**	**6.19**	**6.03**	**5.91**	**5.82**	**5.74**	**5.67**	**5.56**	**5.48**	**5.36**	**5.28**	**5.20**	**5.11**	**5.06**	**5.00**	**4.96**	**4.91**	**4.88**	**4.86**
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.13	3.10	3.07	3 02	2.98	2.93	2 90	2.86	2.82	2 80	2.77	2.76	2.73	2.72	2.71
	10.56	**8.02**	**6.99**	**6.42**	**6.06**	**5.80**	**5.62**	**5.47**	**5.35**	**5.26**	**5.18**	**5.11**	**5.00**	**4.92**	**4.80**	**4.73**	**4.64**	**4.56**	**4.51**	**4.45**	**4.41**	**4.36**	**4.33**	**4.31**

10	4.96	4.10	3.71	3.48	3.33	3 22	3.14	3.07	3.02	2 97	2 94	2 91	2 86	2.82	2.77	2 74	2.70	2.67	2.64	2.61	2.59	2.56	2.55	2.54
	10.04	**7.56**	**6.55**	**5.99**	**5.64**	**5.39**	**5.21**	**5.06**	**4.95**	**4 85**	**4 78**	**4.71**	**4.60**	**4.52**	**4.41**	**4.33**	**4.25**	**4.17**	**4.12**	**4.05**	**4.01**	**3.96**	**3.93**	**3.91**
11	4.84	3.98	3.59	3.36	3 20	3.09	3.01	2.95	2 90	2.86	2 82	2.79	2 74	2.70	2 65	2.61	2.57	2.53	2.50	2.47	2.45	2 42	2.41	2.40
	9.65	**7.20**	**6.22**	**5.67**	**5.32**	**5.07**	**4.88**	**4.74**	**4.63**	**4.54**	**4.46**	**4.40**	**4.29**	**4.21**	**4.10**	**4.02**	**3.94**	**3.86**	**3.80**	**3.74**	**3.70**	**3.66**	**3.62**	**3.60**
12	4.75	3.88	3.49	3 26	3 11	3 00	2 92	2 85	2 80	2 76	2.72	2 69	2 64	2 60	2 54	2.50	2.46	2.42	2.40	2 36	2.35	2.32	2.31	2.30
	9.33	**6.93**	**5.95**	**5.41**	**5.06**	**4.82**	**4.65**	**4.50**	**4.39**	**4.30**	**4.22**	**4.16**	**4.05**	**3.98**	**3.86**	**3.78**	**3.70**	**3.61**	**3.56**	**3.49**	**3.46**	**3.41**	**3.38**	**3.36**
13	4.67	3.80	3 41	3.18	3 02	2 92	2 84	2.77	2 72	2.67	2 63	2 60	2 55	2.51	2 46	2 42	2.38	2 34	2 32	2.28	2.26	2.24	2.22	2.21
	9.07	**6.70**	**5.74**	**5.20**	**4.86**	**4.62**	**4.44**	**4.30**	**4.19**	**4.10**	**4.02**	**3.96**	**3.85**	**3.78**	**3.67**	**3.59**	**3.51**	**3.42**	**3.37**	**3.30**	**3.27**	**3.21**	**3.18**	**3.16**
14	4.60	3.74	3.34	3.11	2 96	2.85	2 77	2 70	2 65	2 60	2 56	2.53	2 48	2.44	2.39	2.35	2.31	2.27	2.24	2.21	2.19	2.16	2 14	2.13
	8.86	**6.51**	**5.56**	**5.03**	**4.69**	**4.46**	**4.28**	**4.14**	**4.03**	**3.94**	**3.86**	**3.80**	**3.70**	**3.62**	**3 51**	**3.43**	**3.34**	**3.26**	**3.21**	**3.14**	**3.11**	**3.06**	**3.02**	**3.00**
15	4 54	3 68	3 29	3.06	2 90	2.79	2 70	2 64	2 59	2.55	2 51	2 48	2 43	2 39	2 33	2 29	2 25	2.21	2 18	2.15	2.12	2.10	2.08	2.07
	8.68	**6.36**	**5.42**	**4.89**	**4.56**	**4.32**	**4.14**	**4.00**	**3.89**	**3.80**	**3.73**	**3.67**	**3.56**	**3.48**	**3.36**	**3.29**	**3.20**	**3.12**	**3.07**	**3.00**	**2.97**	**2.92**	**2.89**	**2.87**
16	4.49	3.63	3.24	3 01	2.85	2 74	2 66	2.59	2 54	2.49	2 45	2.42	2 37	2.33	2.28	2 24	2.20	2 16	2 13	2.09	2.07	2 04	2 02	2.01
	8.53	**6.23**	**5.29**	**4.77**	**4.44**	**4.20**	**4.03**	**3.89**	**3.78**	**3.69**	**3.61**	**3.55**	**3.45**	**3.37**	**3.25**	**3.18**	**3.10**	**3.01**	**2.96**	**2.89**	**2.86**	**2.80**	**2.77**	**2.75**
17	4.45	3.59	3.20	2.96	2.81	2.70	2 62	2 55	2.50	2 45	2.41	2.38	2.33	2 29	2.23	2 19	2 15	2.11	2 08	2.04	2.02	1.99	1.97	1 96
	8.40	**6.11**	**5.18**	**4.67**	**4.34**	**4.10**	**3.93**	**3.79**	**3.68**	**3.59**	**3.52**	**3.45**	**3.35**	**3 27**	**3.16**	**3.08**	**3.00**	**2.92**	**2.86**	**2.79**	**2.76**	**2.70**	**2.67**	**2.65**
18	4.41	3.55	3.16	2.93	2.77	2 66	2.58	2.51	2.46	2.41	2.37	2 34	2 29	2 25	2 19	2.15	2 11	2 07	2.04	2.00	1.98	1.95	1.93	1.92
	8.28	**6.01**	**5.09**	**4.58**	**4.25**	**4.01**	**3.85**	**3.71**	**3.60**	**3.51**	**3.44**	**3.37**	**3.27**	**3.19**	**3.07**	**3.00**	**2.91**	**2.86**	**2.78**	**2.71**	**2.68**	**2.62**	**2.59**	**2.57**
19	4.38	3 52	3 13	2.90	2.74	2 63	2.55	2 48	2 43	2.38	2 34	2 31	2 26	2 21	2 15	2 11	2.07	2.02	2.00	1.96	1.94	1.91	1 90	1.88
	8.18	**5.93**	**5.01**	**4.50**	**4.17**	**3.94**	**3.77**	**3.63**	**3.52**	**3.43**	**3.36**	**3.30**	**3.19**	**3.12**	**3.03**	**2.92**	**2.84**	**2.76**	**2.70**	**2.63**	**2.60**	**2.54**	**2.51**	**2.49**
20	4.35	3.49	3.10	2.87	2.71	2.60	2.52	2.45	2.40	2.35	2.31	2.28	2 23	2 18	2.12	2 08	2.04	1.99	1.96	1.92	1.90	1.87	1.85	1.84
	8.10	**5.85**	**4.94**	**4.43**	**4.10**	**3.87**	**3.71**	**3.56**	**3.45**	**3.37**	**3.30**	**3.23**	**3.13**	**3.05**	**2.94**	**2.86**	**2.77**	**2.69**	**2.63**	**2.56**	**2.53**	**2.47**	**2.44**	**2.42**
21	4.32	3 47	3 07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.28	2 25	2.20	2 15	2.09	2.05	2.00	1 96	1.93	1.89	1.87	1.84	1 82	1.81
	8.02	**5.78**	**4.87**	**4.37**	**4.04**	**3.81**	**3.65**	**3.51**	**3.40**	**3.31**	**3.24**	**3.17**	**3.07**	**2.99**	**2.88**	**2.80**	**2.72**	**2.63**	**2.58**	**2.51**	**2.47**	**2.42**	**2.38**	**2.36**

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TABLE II.—POINTS FOR THE DISTRIBUTION OF F.*—(*Continued*)

Values for 5 per cent in lightface type. Values for 1 per cent in boldface type

n_2	n_1 degrees of freedom (for greater mean square)																						
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
22	4.30	3.44	3.05	2.82	2.66	2.55	2 47	2.40	2.35	2.30	2.26	2.23	2.18	2.13	2 07	2 03	1.98	1.93	1.91	1.87	1 84	1.81	1 80	1.78
	7.94	**5.72**	**4.82**	**4.31**	**3.99**	**3.76**	**3.59**	**3.45**	**3.35**	**3.26**	**3.18**	**3.12**	**3.02**	**2.94**	**2.83**	**2.75**	**2.67**	**2.58**	**2.53**	**2.46**	**2.42**	**2.37**	**2.33**	**2.31**
23	4.28	3 42	3 03	2 80	2.64	2.53	2 45	2.38	2.32	2.28	2 24	2.20	2 14	2 10	2 04	2 00	1.96	1.91	1 88	1.84	1 82	1 79	1.77	1.76
	7.88	**5.66**	**4.76**	**4.26**	**3.94**	**3.71**	**3.54**	**3.41**	**3.30**	**3.21**	**3.14**	**3.07**	**2.97**	**2.89**	**2.78**	**2.70**	**2.62**	**2.53**	**2.48**	**2.41**	**2.37**	**2.32**	**2.28**	**2.26**
24	4.26	3.40	3.01	2.78	2.62	2.51	2 43	2.36	2.30	2.26	2 22	2 18	2 13	2 09	2 02	1 98	1 94	1.89	1.86	1 82	1.80	1.76	1.74	1.73
	7.82	**5.61**	**4.72**	**4.22**	**3.90**	**3.67**	**3.50**	**3.36**	**3.25**	**3.17**	**3.09**	**3.03**	**2.93**	**2.85**	**2.74**	**2.66**	**2.58**	**2.49**	**2.44**	**2.36**	**2.33**	**2.27**	**2.23**	**2.21**
25	4.24	3.38	2.99	2.76	2 60	2.49	2 41	2.34	2.28	2.24	2.20	2.16	2 11	2.06	2 00	1.96	1 92	1 87	1.84	1 80	1.77	1.74	1.72	1.71
	7.77	**5.57**	**4.68**	**4.18**	**3.86**	**3.63**	**3.46**	**3.32**	**3.21**	**3.13**	**3.05**	**2.99**	**2.89**	**2.81**	**2.70**	**2 62**	**2.54**	**2.45**	**2.40**	**2.32**	**2.29**	**2.23**	**2.19**	**2.17**
26	4.22	3.37	2.98	2.74	2 59	2.47	2.39	2 32	2 27	2 22	2 18	2.15	2.10	2.05	1.99	1 95	1.90	1 85	1 82	1.78	1.76	1.72	1.70	1.69
	7.72	**5.53**	**4.64**	**4.14**	**3.82**	**3.59**	**3.42**	**3.29**	**3.17**	**3.09**	**3.02**	**2.96**	**2.86**	**2.77**	**2.66**	**2.58**	**2.50**	**2.41**	**2.36**	**2.28**	**2.25**	**2.19**	**2.15**	**2.13**
27	4.21	3.35	2.96	2.73	2.57	2.46	2.37	2 30	2.25	2.20	2.16	2.13	2 08	2.03	1 97	1 93	1.88	1 84	1.80	1 76	1 74	1.71	1.68	1.67
	7.68	**5.49**	**4.60**	**4.11**	**3.79**	**3.56**	**3.39**	**3.26**	**3.14**	**3.06**	**2.98**	**2.93**	**2.83**	**2.74**	**2.63**	**2.55**	**2.47**	**2.38**	**2.33**	**2.25**	**2.21**	**2.16**	**2.12**	**2.10**
28	4.20	3.34	2.95	2.71	2.56	2.44	2.36	2 29	2.24	2.19	2.15	2 12	2 06	2.02	1.96	1 91	1.87	1.81	1.78	1.75	1.72	1.69	1.67	1.65
	7.64	**5.45**	**4.57**	**4.07**	**3.76**	**3.53**	**3.36**	**3.23**	**3.11**	**3.03**	**2.95**	**2.90**	**2.80**	**2.71**	**2.60**	**2.52**	**2.44**	**2.35**	**2.30**	**2.22**	**2.18**	**2.13**	**2.09**	**2.06**
29	4.18	3.33	2.93	2.70	2.54	2.43	2.35	2 28	2.22	2.18	2 14	2 10	2.05	2 00	1 94	1.90	1 85	1.80	1.77	1.73	1.71	1.68	1.65	1.64
	7.60	**5.42**	**4.54**	**4.04**	**3.73**	**3.50**	**3.33**	**3.20**	**3.08**	**3.00**	**2.92**	**2.87**	**2.77**	**2.68**	**2.57**	**2.49**	**2.41**	**2.32**	**2.27**	**2.19**	**2.15**	**2.10**	**2.06**	**2.03**
30	4.17	3.32	2.92	2.69	2.53	2 42	2 34	2.27	2.21	2.16	2 12	2.09	2.04	1 99	1.93	1.89	1.84	1.79	1.76	1.72	1.69	1 66	1.64	1.62
	7.56	**5.39**	**4.51**	**4.02**	**3.70**	**3.47**	**3.30**	**3.17**	**3.06**	**2.98**	**2.90**	**2.84**	**2.74**	**2.66**	**2.55**	**2.47**	**2.38**	**2.29**	**2.24**	**2.16**	**2.13**	**2.07**	**2.03**	**2.01**

32	4.15	3.30	2.90	2 67	2.51	2.40	2.32	2.25	2.19	2.14	2.10	2.07	2.02	1.97	1.91	1.86	1.82	1.76	1.74	1.69	1.67	1.64	1.61	1.59
	7.50	**5.34**	**4.46**	**3.97**	**3.66**	**3.42**	**3.25**	**3.12**	**3.01**	**2.94**	**2.86**	**2.80**	**2.70**	**2.62**	**2.51**	**2.42**	**2.34**	**2.25**	**2.20**	**2.12**	**2.08**	**2.02**	**1.98**	**1.96**
34	4.13	3.28	2.88	2.65	2.49	2 38	2.30	2.23	2.17	2.12	2 08	2 05	2 00	1.95	1.89	1.84	1 80	1 74	1.71	1.67	1.64	1.61	1.59	1.57
	7.44	**5.29**	**4.42**	**3.93**	**3.61**	**3.38**	**3.21**	**3.08**	**2.97**	**2.89**	**2.82**	**2.76**	**2.66**	**2.58**	**2.47**	**2.38**	**2.30**	**2.21**	**2.15**	**2.08**	**2.04**	**1.98**	**1.94**	**1.91**
36	4.11	3.26	2.86	2.63	2.48	2.36	2.28	2.21	2.15	2 10	2.06	2 03	1 98	1.93	1.87	1 82	1.78	1 72	1.69	1.65	1.62	1.59	1.56	1.55
	7.39	**5.25**	**4.38**	**3.89**	**3.58**	**3.35**	**3.18**	**3.04**	**2.94**	**2.86**	**2.78**	**2.72**	**2.62**	**2.54**	**2.43**	**2.35**	**2.26**	**2.17**	**2.12**	**2.04**	**2.00**	**1.94**	**1.90**	**1.87**
38	4.10	3.25	2.85	2.62	2.46	2.35	2.26	2.19	2 14	2 09	2 05	2 02	1 96	1 92	1 85	1.80	1 76	1.71	1.67	1.63	1.60	1.57	1.54	1.53
	7.35	**5.21**	**4.34**	**3.86**	**3.54**	**3.32**	**3.15**	**3.02**	**2.91**	**2.82**	**2.75**	**2.69**	**2.59**	**2.51**	**2.40**	**2.32**	**2.22**	**2.14**	**2.08**	**2.00**	**1.97**	**1.90**	**1.86**	**1.84**
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.07	2 04	2 00	1.95	1.90	1 84	1.79	1.74	1 69	1.66	1.61	1.59	1.55	1.53	1.51
	7.31	**5.18**	**4.31**	**3.83**	**3.51**	**3.29**	**3.12**	**2.99**	**2.88**	**2.80**	**2.73**	**2.66**	**2.56**	**2.49**	**2.37**	**2.29**	**2.20**	**2.11**	**2.05**	**1.97**	**1.94**	**1.88**	**1.84**	**1.81**
42	4.07	3.22	2.83	2.59	2.44	2.32	2.24	2.17	2.11	2 06	2.02	1.99	1.94	1.89	1 82	1.78	1.73	1 68	1.64	1.60	1.57	1.54	1.51	1.49
	7.27	**5.15**	**4.29**	**3.80**	**3.49**	**3.26**	**3.10**	**2.96**	**2.86**	**2.77**	**2.70**	**2.64**	**2.54**	**2.46**	**2.35**	**2.26**	**2.17**	**2.08**	**2.02**	**1.94**	**1.91**	**1.85**	**1.80**	**1.78**
44	4.06	3.21	2 82	2 58	2.43	2.31	2.23	2.16	2.10	2 05	2 01	1 98	1 92	1.88	1 81	1 76	1.72	1.66	1.63	1.58	1.56	1.52	1.50	1.48
	7.24	**5.12**	**4.26**	**3.78**	**3.46**	**3.24**	**3.07**	**2.94**	**2.84**	**2.75**	**2.68**	**2.62**	**2.52**	**2.44**	**2.32**	**2.24**	**2.15**	**2.06**	**2.00**	**1.92**	**1.88**	**1.82**	**1.78**	**1.75**
46	4.05	3.20	2.81	2.57	2.42	2.30	2 22	2.14	2.09	2 04	2.00	1.97	1.91	1.87	1.80	1 75	1 71	1.65	1.62	1.57	1.54	1.51	1.48	1.46
	7.21	**5.10**	**4.24**	**3.76**	**3.44**	**3.22**	**3.05**	**2.92**	**2.82**	**2.73**	**2.66**	**2.60**	**2.50**	**2.42**	**2.30**	**2.22**	**2.13**	**2.04**	**1.98**	**1.90**	**1.86**	**1.80**	**1.76**	**1.72**
48	4.04	3.19	2 80	2.56	2.41	2.30	2 21	2.14	2 08	2.03	1.99	1.96	1.90	1.86	1.79	1.74	1.70	1.64	1.61	1 56	1.53	1.50	1.47	1.45
	7.19	**5.08**	**4.22**	**3.74**	**3.42**	**3.20**	**3.04**	**2.90**	**2.80**	**2.71**	**2.64**	**2.58**	**2.48**	**2.40**	**2.28**	**2.20**	**2.11**	**2.02**	**1.96**	**1.88**	**1.84**	**1.78**	**1.73**	**1.70**
50	4.03	3.18	2.79	2.56	2.40	2.29	2.20	2.13	2 07	2 02	1.98	1.95	1.90	1.85	1.78	1 74	1.69	1.63	1.60	1 55	1.52	1.48	1.46	1.44
	7.17	**5.06**	**4.20**	**3.72**	**3.41**	**3.18**	**3.02**	**2.88**	**2.78**	**2.70**	**2.62**	**2.56**	**2.46**	**2.39**	**2.26**	**2.18**	**2.10**	**2.00**	**1.94**	**1.86**	**1.82**	**1.76**	**1.71**	**1.68**
55	4.02	3.17	2.78	2.54	2.38	2.27	2.18	2.11	2.05	2.00	1.97	1.93	1.88	1.83	1.76	1.72	1 67	1.61	1.58	1.52	1.50	1.46	1.43	1.41
	7.12	**5.01**	**4.16**	**3.68**	**3.37**	**3.15**	**2.98**	**2.85**	**2.75**	**2.66**	**2.59**	**2.53**	**2.43**	**2.35**	**2.23**	**2.15**	**2.06**	**1.96**	**1.90**	**1.82**	**1.78**	**1.71**	**1.66**	**1.64**
60	4.00	3.15	2.76	2.52	2.37	2.25	2.17	2.10	2.04	1.99	1.95	1.92	1 86	1.81	1.75	1.70	1.65	1.59	1.56	1.50	1.48	1.44	1.41	1.39
	7.08	**4.98**	**4.13**	**3.65**	**3.34**	**3.12**	**2.95**	**2.82**	**2.72**	**2.63**	**2.56**	**2.50**	**2.40**	**2.32**	**2.20**	**2.12**	**2.03**	**1.93**	**1.87**	**1.79**	**1.74**	**1.68**	**1.63**	**1.60**

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TABLE II.—POINTS FOR THE DISTRIBUTION OF F.*—(*Continued*)

Values for 5 per cent in lightface type. Values for 1 per cent in boldface type

n_2	n_1 degrees of freedom (for greater mean square)																						
	1	2	3	4	5	6	7	8	9	10	11	12	14	16	20	24	30	40	50	75	100	200	500	∞
65	3.99	3.14	2.75	2.51	2.36	2.24	2.15	2.08	2.02	1.98	1 94	1.90	1.85	1.80	1 73	1.68	1 63	1.57	1.54	1.49	1.46	1.42	1.39	1.37
	7.04	**4.95**	**4.10**	**3.62**	**3.31**	**3.09**	**2.93**	**2.79**	**2.70**	**2.61**	**2.54**	**2.47**	**2.37**	**2.30**	**2.18**	**2.09**	**2.00**	**1.90**	**1.84**	**1.76**	**1.71**	**1.64**	**1.60**	**1.56**
70	3.98	3.13	2.74	2.50	2.35	2.23	2.14	2.07	2.01	1.97	1.93	1.89	1.84	1 79	1.72	1.67	1.62	1.56	1.53	1.47	1.45	1.40	1.37	1.35
	7.01	**4.92**	**4.08**	**3.60**	**3.29**	**3.07**	**2.91**	**2.77**	**2.67**	**2.59**	**2.51**	**2.45**	**2.35**	**2.28**	**2.15**	**2.07**	**1.98**	**1.88**	**1.82**	**1.74**	**1.69**	**1.62**	**1.56**	**1.53**
80	3.96	3.11	2.72	2.48	2.33	2.21	2 12	2.05	1.99	1.95	1.91	1.88	1.82	1.77	1.70	1.65	1.60	1.54	1.51	1.45	1.42	1.38	1.35	1.32
	6.96	**4.88**	**4.04**	**3.56**	**3.25**	**3.04**	**2.87**	**2.74**	**2.64**	**2.55**	**2.48**	**2.41**	**2.32**	**2.24**	**2.11**	**2.03**	**1.94**	**1.84**	**1.78**	**1.70**	**1.65**	**1.57**	**1.52**	**1.49**
100	3.94	3 09	2.70	2 46	2.30	2 19	2 10	2.03	1.97	1 92	1 88	1.85	1.79	1.75	1 68	1 63	1.57	1.51	1.48	1.42	1.39	1.34	1.30	1.28
	6.90	**4.82**	**3.98**	**3.51**	**3.20**	**2.99**	**2.82**	**2.69**	**2.59**	**2.51**	**2.43**	**2.36**	**2.26**	**2.19**	**2.06**	**1.98**	**1.89**	**1.79**	**1.73**	**1.64**	**1.59**	**1.51**	**1.46**	**1.43**
125	3.92	3.07	2.68	2.44	2.29	2.17	2 08	2 01	1.95	1.90	1.86	1.83	1 77	1.72	1 65	1.60	1.55	1.49	1.45	1.39	1.36	1.31	1.27	1.25
	6.84	**4.78**	**3.94**	**3.47**	**3.17**	**2.95**	**2.79**	**2.65**	**2.56**	**2.47**	**2.40**	**2.33**	**2.23**	**2.15**	**2.03**	**1.94**	**1.85**	**1.75**	**1.68**	**1.59**	**1.54**	**1.46**	**1.40**	**1.37**
150	3.91	3.06	2.67	2.43	2.27	2.16	2.07	2.00	1.94	1.89	1.85	1.82	1.76	1.71	1.64	1.59	1.54	1.47	1.44	1.37	1.34	1.29	1.25	1.22
	6.81	**4.75**	**3.91**	**3.44**	**3.14**	**2.92**	**2.76**	**2.62**	**2.53**	**2.44**	**2.37**	**2.30**	**2.20**	**2.12**	**2.00**	**1.91**	**1.83**	**1.72**	**1.66**	**1.56**	**1.51**	**1.43**	**1.37**	**1.33**
200	3.89	3 04	2.65	2.41	2 26	2.14	2.05	1 98	1 92	1.87	1.83	1 80	1.74	1.69	1 62	1.57	1 52	1.45	1.42	1.35	1.32	1.26	1.22	1.19
	6.76	**4.71**	**3.88**	**3.41**	**3.11**	**2.90**	**2.73**	**2.60**	**2.50**	**2.41**	**2.34**	**2.28**	**2.17**	**2.09**	**1.97**	**1.88**	**1.79**	**1.69**	**1.62**	**1.53**	**1.48**	**1.39**	**1.33**	**1.28**
400	3.86	3.02	2.62	2.39	2.23	2.12	2 03	1 96	1.90	1.85	1.81	1.78	1.72	1.67	1.60	1.54	1.49	1.42	1.38	1.32	1.28	1.22	1.16	1.13
	6.70	**4.66**	**3.83**	**3.36**	**3.06**	**2.85**	**2.69**	**2.55**	**2.46**	**2.37**	**2.29**	**2.23**	**2.12**	**2.04**	**1.92**	**1.84**	**1.74**	**1.64**	**1.57**	**1.47**	**1.42**	**1.32**	**1.24**	**1.19**
1000	3.85	3.00	2.61	2.38	2.22	2.10	2.02	1.95	1.89	1.84	1.80	1.76	1.70	1.65	1.58	1.53	1.47	1.41	1.36	1.30	1.26	1.19	1.13	1.08
	6.66	**4.62**	**3.80**	**3.34**	**3.04**	**2.82**	**2.66**	**2.53**	**2.43**	**2.34**	**2.26**	**2.20**	**2.09**	**2.01**	**1.89**	**1.81**	**1.71**	**1.61**	**1.54**	**1.44**	**1.38**	**1.28**	**1.19**	**1.11**
∞	3.84	2 99	2.60	2 37	2 21	2.09	2.01	1.94	1.88	1.83	1 79	1.75	1.69	1.64	1 57	1.52	1.46	1 40	1.35	1.28	1.24	1.17	1.11	1.00
	6.64	**4.60**	**3.78**	**3.32**	**3.02**	**2.80**	**2.64**	**2.51**	**2.41**	**2.32**	**2.24**	**2.18**	**2.07**	**1.99**	**1.87**	**1.79**	**1.69**	**1.59**	**1.52**	**1.41**	**1.36**	**1.25**	**1.15**	**1.00**

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TABLE III.—TABLE OF χ^2*

Degrees of freedom	Probability (P)							
	.99	.95	.50	.20	.10	.05	.02	.01
1	0.0002	0.004	0.46	1 64	2.71	3.84	5.41	6.64
2	0 020	0 103	1.39	3.22	4.60	5.99	7.82	9.21
3	0.115	0.35	2.37	4.64	6.25	7.82	9.84	11 34
4	0.30	0.71	3.36	5.99	7.78	9.49	11.67	13.28
5	0.55	1.14	4.35	7.29	9.24	11.07	13.39	15.09
6	0 87	1.64	5.35	8.56	10.64	12 59	15.03	16.81
7	1 24	2.17	6.35	9.80	12.02	14.07	16.62	18 48
8	1.65	2.73	7.34	11.03	13 36	15.51	18 17	20.09
9	2 09	3.32	8.34	12.24	14.68	16 92	19.68	21.67
10	2.56	3.94	9.34	13.44	15.99	18.31	21.16	23.21
11	3.05	4.58	10.34	14.63	17.28	19 68	22 62	24.72
12	3.57	5.23	11.34	15.81	18 55	21.03	24.05	26.22
13	4 11	5 89	12 34	16 98	19 81	22 36	25 47	27 69
14	4.66	6.57	13 34	18.15	21 06	23 68	26 87	29 14
15	5 23	7.26	14.34	19 31	22.31	25 00	28 26	30.58
16	5 81	7.96	15.34	20.46	23.54	26.30	29.63	32.00
17	6.41	8.67	16.34	21.62	24.77	27.59	31 00	33.41
18	7 02	9 39	17 34	22.76	25.99	28.87	32 35	34.80
19	7.63	10 12	18.34	23.90	27.20	30.14	33.69	36.19
20	8.26	10 85	19.34	25.04	28.41	31.41	35.02	37.57
21	8.90	11 59	20.34	26.17	29.62	32.67	36.34	38.93
22	9.54	12 34	21.34	27.30	30.81	33.92	37.66	40 29
23	10 20	13 09	22.34	28.43	32 01	35.17	38.97	41.64
24	10.86	13.85	23.34	29.55	33.20	36.42	40.27	42.98
25	11 52	14.61	24.34	30.68	34.38	37.65	41 57	44.31
26	12.20	15.38	25.34	31.80	35.56	38.88	42.86	45.64
27	12.88	16 15	26.34	32.91	36.74	40.11	44.14	46 96
28	13.56	16 93	27 34	34.03	37.92	41.34	45.42	48.28
29	14.26	17.71	28.34	35.14	39.09	42.56	46.69	49.59
30	14.95	18.49	29.34	36.25	40.26	43.77	47.96	50.89

For larger values of n, the expression $\sqrt{2\chi^2} - \sqrt{2n-1}$ may be used as a normal deviate with unit variance.

* Abridged from Table III of Fisher's "Statistical Methods for Research Workers," Oliver & Boyd, Edinburgh, by kind permission of the author and publishers.

TABLE IV.—TABLE OF r, FOR VALUES OF z FROM 0 TO 3*

z	.01	.02	.03	.04	.05	.06	.07	.08	.09	.10
0 0	0100	0200	.0300	.0400	.0500	.0599	.0699	.0798	.0898	0997
0 1	.1096	.1194	.1293	.1391	.1489	.1586	.1684	.1781	.1877	1974
0 2	2070	2165	2260	.2355	2449	2543	2636	2729	.2821	2913
0.3	3004	.3095	3185	.3275	3364	3452	3540	.3627	.3714	3800
0 4	3885	3969	4053	4136	1219	.4301	.4382	4462	.4542	4621
0 5	4699	4777	.4854	.4930	.5005	.5080	.5154	.5227	.5299	5370
0 6	5441	5511	5580	.5649	.5717	5784	5850	.5915	.5980	.6044
0 7	6107	.6169	6231	.6291	6351	.6411	6469	.6527	6584	6640
0 8	6696	6751	6805	6858	.6911	6963	.7014	.7064	.7114	7163
0 9	7211	7259	.7306	7352	.7398	.7443	.7487	.7531	7574	7616
1 0	7658	7699	.7739	7779	7818	7857	.7895	.7932	7969	8005
1 1	8041	8076	8110	8144	8178	8210	8243	.8275	.8306	8337
1 2	8367	8397	8426	8455	.8483	.8511	.8538	.8565	.8591	8617
1.3	8643	8668	.8692	.8717	.8741	8764	.8787	.8810	.8832	8854
1 4	8875	8896	8917	8937	8957	.8977	8996	.9015	9033	.9051
1 5	9069	9087	9104	.9121	9138	9154	9170	9186	.9201	9217
1 6	9232	9246	9261	.9275	.9289	9302	9316	9329	.9341	9354
1 7	9366	.9379	.9391	.9102	.9414	9425	9436	.9447	9458	94681
1 8	94783	94884	91983	95080	.95175	95268	95359	.95449	95537	95624
1 9	95709	95792	95873	95953	.96032	96109	96185	.96259	.96331	96403
2.0	96473	96541	96609	96675	96739	96803	.96865	.96926	96986	97045
2.1	97103	97159	97215	97269	97323	.97375	.97426	.97477	.97526	.97574
2 2	97622	97668	97714	97759	97803	97846	97888	97929	.97970	98010
2 3	98049	98087	98124	.98161	98197	.98233	98267	98301	98335	98367
2 4	98399	98431	98462	.98492	98522	.98551	.98579	.98607	98635	.98661
2.5	98688	.98714	98739	98764	98788	98812	98835	.98858	.98881	98903
2 6	98924	98945	98966	98987	.99007	99026	.99045	99064	.99083	99101
2.7	99118	99136	99153	.99170	99186	99202	99218	.99233	99248	.99263
2 8	99278	.99292	.99306	99320	99333	99346	.99359	.99372	.99384	.99396
2.9	.99408	99420	99431	99443	.99454	.99464	99475	99485	.99195	.99505

For greater accuracy, and for values beyond the table, $r = (e^{2z} - 1) \div (e^{2z} + 1)$; $z = \frac{1}{2}\{\log (1 + r) - \log (1 - r)\}$

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TABLE V.—SIGNIFICANT VALUES OF r AND R.*

Values for $P = .05$ in lightface type. Values for $P = .01$ in boldface type

Degrees of freedom	Number of variables								
	2	3	4	5	6	7	9	13	25
1	997 **1.000**	999 **1.000**	999 **1.000**	.999 **1.000**	1 000 **1.000**	1 000 **1.000**	1 000 **1.000**	1 000 **1.000**	1 000 **1.000**
2	950 **.990**	975 **.995**	983 **.997**	.987 **.998**	990 **.998**	992 **.998**	994 **.999**	996 **.999**	998 **1.000**
3	.878 **.959**	930 **.976**	950 **.983**	961 **.987**	.968 **.990**	973 **.991**	979 **.993**	986 **.995**	993 **.998**
4	.811 **.917**	881 **.949**	.912 **.962**	.930 **.970**	.942 **.975**	950 **.979**	961 **.984**	973 **.989**	986 **.994**
5	.754 **.874**	836 **.917**	874 **.937**	898 **.949**	.914 **.957**	.925 **.963**	.941 **.971**	958 **.980**	978 **.989**
6	707 **.834**	795 **.886**	839 **.911**	867 **.927**	.886 **.938**	.900 **.946**	920 **.957**	943 **.969**	969 **.983**
7	666 **.798**	758 **.855**	807 **.885**	838 **.904**	860 **.918**	876 **.928**	900 **.942**	927 **.958**	960 **.977**
8	632 **.765**	726 **.827**	777 **.860**	811 **.882**	835 **.898**	854 **.909**	880 **.926**	912 **.946**	950 **.970**
9	602 **.735**	697 **.800**	750 **.836**	.786 **.861**	812 **.878**	832 **.891**	.861 **.911**	.897 **.934**	941 **.963**
10	576 **.708**	671 **.776**	726 **.814**	763 **.840**	.790 **.859**	812 **.874**	843 **.895**	882 **.922**	932 **.955**
11	553 **.684**	648 **.753**	703 **.793**	741 **.821**	.770 **.841**	792 **.857**	826 **.880**	868 **.910**	922 **.948**
12	532 **.661**	627 **.732**	683 **.773**	722 **.802**	751 **.824**	774 **.841**	.809 **.866**	.854 **.898**	913 **.940**
13	514 **.641**	608 **.712**	.664 **.755**	703 **.785**	.733 **.807**	.757 **.825**	.794 **.852**	.840 **.886**	904 **.932**
14	497 **.623**	590 **.694**	.646 **.737**	.686 **.768**	.717 **.792**	741 **.810**	779 **.838**	.828 **.875**	.895 **.924**
15	482 **.606**	574 **.677**	.630 **.721**	.670 **.752**	.701 **.776**	726 **.796**	765 **.825**	815 **.864**	886 **.917**
16	.468 **.590**	559 **.662**	.615 **.706**	655 **.738**	.686 **.762**	712 **.782**	751 **.813**	.803 **.853**	.878 **.909**
17	.456 **.575**	545 **.647**	.601 **.691**	.641 **.724**	.673 **.749**	.698 **.769**	738 **.800**	792 **.842**	869 **.902**
18	.444 **.561**	.532 **.633**	.587 **.678**	.628 **.710**	.660 **.736**	.686 **.756**	.726 **.789**	781 **.832**	861 **.894**
19	.433 **.549**	.520 **.620**	.575 **.665**	.615 **.698**	.647 **.723**	.674 **.744**	.714 **.778**	.770 **.822**	853 **.887**
20	.423 **.537**	509 **.608**	.563 **.652**	.604 **.685**	.636 **.712**	.662 **.733**	.703 **.767**	.760 **.812**	.845 **.880**
21	.413 **.526**	198 **.596**	.552 **.641**	.592 **.674**	.624 **.700**	.651 **.722**	.693 **.756**	.750 **.803**	.837 **.873**
22	404 **.515**	188 **.585**	542 **.630**	582 **.663**	.614 **.690**	640 **.712**	.682 **.746**	.740 **.794**	.830 **.866**
23	.396 **.505**	.479 **.574**	532 **.619**	.572 **.652**	604 **.679**	.630 **.701**	673 **.736**	.731 **.785**	.823 **.859**
24	.388 **.496**	.470 **.565**	523 **.609**	.562 **.642**	.594 **.669**	.621 **.692**	.663 **.727**	.722 **.776**	.815 **.852**

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TABLE V.—SIGNIFICANT VALUES OF r AND R.*—(*Continued*)

Values for P = .05 in lightface type. Values for P = .01 in boldface type

Degrees of freedom	Number of variables								
	2	3	4	5	6	7	9	13	25
25	.381 **.487**	.462 **.555**	.514 **.600**	.553 **.633**	.585 **.660**	.612 **.682**	.654 **.718**	.714 **.768**	.808 **.846**
26	.374 **.478**	.454 **.546**	.506 **.590**	.545 **.624**	.576 **.651**	.603 **.673**	.645 **.709**	.706 **.760**	.802 **.839**
27	.367 **.470**	.446 **.538**	.498 **.582**	.536 **.615**	.568 **.642**	.594 **.664**	.637 **.701**	.698 **.752**	.795 **.833**
28	.361 **.463**	.439 **.530**	.490 **.573**	.529 **.606**	.560 **.634**	.586 **.656**	.629 **.692**	.690 **.744**	.788 **.827**
29	.355 **.456**	.432 **.522**	.482 **.565**	.521 **.598**	.552 **.625**	.579 **.648**	.621 **.685**	.682 **.737**	.782 **.821**
30	.349 **.449**	.426 **.514**	.476 **.558**	.514 **.591**	.545 **.618**	.571 **.640**	.614 **.677**	.675 **.729**	.776 **.815**
35	.325 **.418**	.397 **.481**	.445 **.523**	.482 **.556**	.512 **.582**	.538 **.605**	.580 **.642**	.642 **.696**	.746 **.786**
40	.304 **.393**	.373 **.454**	.419 **.494**	.455 **.526**	.484 **.552**	.509 **.575**	.551 **.612**	.613 **.667**	.720 **.761**
45	.288 **.372**	.353 **.430**	.397 **.470**	.432 **.501**	.460 **.527**	.485 **.549**	.526 **.586**	.587 **.640**	.696 **.737**
50	.273 **.354**	.336 **.410**	.379 **.449**	.412 **.479**	.440 **.504**	.464 **.526**	.504 **.562**	.565 **.617**	.674 **.715**
60	.250 **.325**	.308 **.377**	.348 **.414**	.380 **.442**	.406 **.466**	.429 **.488**	.467 **.523**	.526 **.577**	.636 **.677**
70	.232 **.302**	.286 **.351**	.324 **.386**	.354 **.413**	.379 **.436**	.401 **.456**	.438 **.491**	.495 **.544**	.604 **.644**
80	.217 **.283**	.269 **.330**	.304 **.362**	.332 **.389**	.356 **.411**	.377 **.431**	.413 **.464**	.469 **.516**	.576 **.615**
90	.205 **.267**	.254 **.312**	.288 **.343**	.315 **.368**	.338 **.390**	.358 **.409**	.392 **.441**	.446 **.492**	.552 **.590**
100	.195 **.254**	.241 **.297**	.274 **.327**	.300 **.351**	.322 **.372**	.341 **.390**	.374 **.421**	.426 **.470**	.530 **.568**
125	.174 **.228**	.216 **.266**	.246 **.294**	.269 **.316**	.290 **.335**	.307 **.352**	.338 **.381**	.387 **.428**	.485 **.521**
150	.159 **.208**	.198 **.244**	.225 **.270**	.247 **.290**	.266 **.308**	.282 **.324**	.310 **.351**	.356 **.395**	.450 **.484**
200	.138 **.181**	.172 **.212**	.196 **.234**	.215 **.253**	.231 **.269**	.246 **.283**	.271 **.307**	.312 **.347**	.398 **.430**
300	.113 **.148**	.141 **.174**	.160 **.192**	.176 **.208**	.190 **.221**	.202 **.233**	.223 **.253**	.258 **.287**	.332 **.359**
400	.098 **.128**	.122 **.151**	.139 **.167**	.153 **.180**	.165 **.192**	.176 **.202**	.194 **.220**	.225 **.250**	291 **.315**
500	.088 **.115**	.109 **.135**	.124 **.150**	.137 **.162**	.148 **.172**	.157 **.182**	.174 **.198**	.202 **.225**	.262 **.284**
1000	.062 **.081**	.077 **.096**	.088 **.106**	.097 **.115**	.105 **.122**	.112 **.129**	.124 **.141**	.144 **.160**	.188 **.204**

For total and partial correlation coefficients, use column for 2 variables in table for 5 and 1 per cent points. Degrees of freedom = (number of observations) − (number of variables).

For multiple-correlation coefficients, use the column corresponding to the number of variables. Degrees of freedom = (number of observations) − (number of variables).

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TABLE VI.—TRANSFORMATION OF PERCENTAGE TO DEGREES
Percentage (p) = $\sin^2 \theta$*

Per cent	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.0	0	1 8	2 6	3 1	3 6	4 1	4 4	4.8	5 1	5 4
1	5 7	6 0	6 3	6.5	6 8	7.0	7 3	7 5	7 7	7 9
2	8 1	8.3	8 5	8 7	8 9	9 1	9 3	9 5	9.6	9 8
3	10.0	10.1	10.3	10.5	10.6	10.8	10.9	11.1	11.2	11 4
4	11.5	11.7	11.8	12.0	12.1	12.2	12 4	12.5	12.7	12 8
5	12 9	13 1	13 2	13.3	13.4	13.6	13.7	13.8	13 9	14 1
6	14 2	14.3	14.4	14 5	14 7	14.8	14 9	15.0	15 1	15 2
7	15 3	15 5	15 6	15 7	15 8	15 9	16 0	16 1	16 2	16 3
8	16 4	16 5	16 6	16 7	16 8	17 0	17.1	17.2	17 3	17 4
9	17.5	17.6	17.7	17 8	17 9	18 0	18 0	18.1	18 2	18 3
10	18.4	18 5	18.6	18.7	18.8	18.9	19.0	19.1	19.2	19 3
11	19 4	19 5	19.6	19.6	19.7	19.8	19.9	20 0	20.1	20.2
12	20 3	20 4	20.4	20.5	20.6	20.7	20 8	20.9	21.0	21.0
13	21 1	21 2	21.3	21 4	21.5	21.6	21.6	21.7	21.8	21.9
14	22 0	22 1	22 1	22.2	22.3	22 4	22.5	22 5	22.6	22 7
15	22 8	22 9	22 9	23.0	23.1	23.2	23.3	23 3	23.4	23.5
16	23 6	23 7	23 7	23.8	23.9	24.0	24 0	24.1	24.2	24 3
17	24 4	24 4	24.5	24 6	24.7	24.7	24.8	24.9	25 0	25 0
18	25 1	25.2	25 3	25.3	25.4	25.5	25 5	25 6	25 7	25 8
19	25 8	25 9	26 0	26 1	26.1	26.2	26 3	26.3	26 4	26 5
20	26 6	26 6	26 7	26.8	26.9	26.9	27.0	27.1	27.1	27 2
21	27 3	27 3	27.4	27 5	27.6	27 6	27.7	27 8	27.8	27 9
22	28 0	28 0	28 1	28.2	28.2	28 3	28 4	28.5	28 5	28 6
23	28.7	28.7	28 8	28 9	28.9	29.0	29 1	29 1	29 2	29 3
24	29 3	29.4	29.5	29 5	29.6	29.7	29.7	29.8	29.9	29.9
25	30 0	30.1	30.1	30.2	30.3	30.3	30.4	30.5	30.5	30.6
26	30.7	30 7	30.8	30.9	30.9	31.0	31.0	31.1	31.2	31 2
27	31 3	31.4	31.4	31.5	31.6	31.6	31.7	31.8	31.8	31 9
28	31 9	32.0	32 1	32 1	32.2	32 3	32.3	32 4	32.5	32 5
29	32.6	32 6	32.7	32.8	32.8	32.9	33.0	33.0	33.1	33 1
30	33 2	33.3	33.3	33.4	33.5	33.5	33.6	33.6	33.7	33 8
31	33 8	33.9	34.0	34.0	34.1	34.1	34 2	34.3	34.3	34 4
32	34 4	34.5	34 6	34.6	34.7	34.8	34.8	34.9	34.9	35 0
33	35.1	35.1	35.2	35 2	35.3	35.4	35.4	35.5	35.5	35 6
34	35 7	35 7	35.8	35 8	35.9	36.0	36 0	36.1	36.2	36 2
35	36.3	36.3	36 4	36.5	36.5	36 6	36 6	36.7	36.8	36 8

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TABLE VI.—TRANSFORMATION OF PERCENTAGE TO DEGREES.—(*Continued*)

Per cent	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
36	36 9	36 9	37 0	37 0	37 1	37 2	37 2	37 3	37 3	37 4
37	37 5	37 5	37.6	37.6	37.7	37.8	37.8	37.9	37.9	38.0
38	38 1	38 1	38 2	38 2	38 3	38 4	38.4	38.5	38.5	38.6
39	38.6	38 7	38 8	38.8	38 9	38 9	39.0	39.1	39 1	39 2
40	39.2	39 3	39.3	39 4	39.5	39.5	39.6	39 6	39.7	39.8
41	39 8	39 9	39 9	40.0	40.0	40 1	40.2	40.2	40 3	40.3
42	40 4	40 5	40 5	40 6	40 6	40.7	40.7	40.8	40 9	40 9
43	41 0	41 0	41 1	41 1	41.2	41 3	41 3	41 4	41 4	41 5
44	41 6	41 6	41 7	41 7	41.8	41.8	41 9	42 0	42.0	42 1
45	42.1	42.2	42 2	42.3	42 4	42 4	42 5	42 5	42 6	42 6
46	42 7	42 8	42 8	42 9	42.9	43 0	43 0	43.1	43 2	43 2
47	43 3	43 3	43 4	43 5	43 5	43 6	43 6	43 7	43 7	43 8
48	43 9	43 9	44 0	44 0	44 1	44 1	44 2	44 3	44 3	44 4
49	44 4	44 5	44 5	44 6	44 7	44.7	44.8	44 8	44 9	44 9
50	45 0	45.1	45.1	45.2	45.2	45 3	45 3	45 4	45 5	45 5
51	45.6	45 6	45.7	45.7	45 8	45 9	45 9	46.0	46 0	46 1
52	46 1	46 2	46 3	46 3	46.4	46.4	46.5	46.5	46 6	46 7
53	46 7	46 8	46 8	46 9	47 0	47 0	47.1	47 1	47 2	47 2
54	47.3	47 4	47.4	47.5	47 5	47 6	47.6	47 7	47 8	47 8
55	47.9	47 9	48 0	48.0	48.1	48.2	48.2	48.3	48.3	48 4
56	48 4	48 5	48 6	48.6	48.7	48 7	48 8	48.9	48 9	49 0
57	49 0	49 1	49 1	49 2	49.3	49.3	49 4	49.4	49.5	49 5
58	49 6	49 7	49 7	49.8	49 8	49 9	50.0	50.0	50.1	50 1
59	50 2	50 2	50 3	50 4	50.4	50.5	50.5	50 6	50.7	50.7
60	50.8	50.8	50 9	50.9	51.0	51.1	51.1	51.2	51.2	51 3
61	51 4	51 4	51.5	51 5	51.6	51.6	51.7	51.8	51.8	51.9
62	51 9	52 0	52 1	52.1	52.2	52 2	52.3	52.4	52.4	52.5
63	52 5	52 6	52 7	52 7	52.8	52.8	52.9	53.0	53.0	53 1
64	53.1	53.2	53.2	53 3	53.4	53.4	53.5	53.5	53 6	53 7
65	53.7	53 8	53.8	53.9	54.0	54.0	54.1	54.2	54.2	54.3
66	54.3	54 4	54 5	54.5	54 6	54 6	54.7	54.8	54.8	54.9
67	54 9	55.0	55 1	55 1	55 2	55 2	55.3	55.4	55 4	55.5
68	55 6	55.6	55 7	55.7	55 8	55 9	55 9	56 0	56.0	56 1
69	56.2	56 2	56.3	56.4	56.4	56.5	56.5	56.6	56.7	56.7
70	56.8	56.9	56.9	57.0	57.0	57.1	57.2	57.2	57.3	57.4

TABLE VI.—TRANSFORMATION OF PERCENTAGE TO DEGREES.—(*Continued*)

Per cent	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
71	57.4	57.5	57.5	57.6	57.7	57.7	57.8	57.9	57 9	58 0
72	58.1	58.1	58.2	58.2	58 3	58.4	58.4	58.5	58.6	58 6
73	58 7	58.8	58 8	58 9	59 0	59.0	59 1	59.1	59.2	59 3
74	59 3	59 4	59.5	59 5	59.6	59.7	59.7	59.8	59 9	59 9
75	60 0	60 1	60.1	60 2	60.3	60 3	60.4	60.5	60 5	60 6
76	60 7	60.7	60.8	60.9	60 9	61.0	61.1	61.1	61 2	61 3
77	61 3	61 4	61 5	61 5	61 6	61.7	61 8	61 8	61.9	62 0
78	62 0	62.1	62.2	62 2	62.3	62 4	62 4	62.5	62.6	62.7
79	62 7	62.8	62.9	62.9	63.0	63.1	63 1	63.2	63 3	63 4
80	63.4	63 5	63.6	63.7	63.7	63.8	63 9	63 9	64 0	64 1
81	64.2	64.2	64.3	64.4	64.5	64.5	64.6	64.7	64.7	64 8
82	64 9	65 0	65 0	65 1	65.2	65 3	65.3	65 4	65 5	65 6
83	65 6	65.7	65 8	65.9	66.0	66 0	66 1	66.2	66 3	66 3
84	66 4	66.5	66 6	66 7	66.7	66.8	66.9	67.0	67.1	67 1
85	67 2	67.3	67 4	67 5	67.5	67.6	67 7	67 8	67.9	67.9
86	68 0	68 1	68 2	68 3	68 4	68.4	68.5	68 6	68 7	68.8
87	68 9	69 0	69 0	69 1	69 2	69 3	69 4	69 5	69.6	69 6
88	69 7	69.8	69 9	70 0	70 1	70.2	70 3	70.4	70.4	70 5
89	70 6	70 7	70 8	70 9	71.0	71 1	71.2	71.3	71 4	71 5
90	71 6	71 7	71.8	71 9	72.0	72 0	72.1	72.2	72.3	72 4
91	72 5	72 6	72 7	72 8	72.9	73.0	73 2	73 3	73 4	73 5
92	73 6	73 7	73 8	73 9	74.0	74 1	74 2	74 3	74 4	74 5
93	74.7	74 8	74 9	75 0	75.1	75.2	75 3	75 5	75 6	75.7
94	75 8	75 9	76 1	76 2	76.3	76.4	76.6	76 7	76.8	76 9
95	77.1	77 2	77.3	77.5	77.6	77.8	77.9	78.0	78 2	78 3
96	78.5	78 6	78.8	78 9	79.1	79 2	79.4	79.5	79.7	79 9
97	80.0	80.2	80.4	80 5	80 7	80.9	81.1	81.3	81.5	81.7
98	81.9	82.1	82 3	82 5	82 7	83 0	83 2	83 5	83 7	84 0
99	84.3	84.6	84.9	85.2	85 6	85 9	86 4	86 9	87 4	88 2
100	90 0									

INDEX

A

Aamodt, 134–136, 138
Aase, 20
Ahlgren, 185
Alexander, 188
Alfalfa, effects of self-fertilization, 243–247
  inflorescence, 68–69
  selfing and crossing, 69
Allard, 85
Anderson, 203
Arny, 170, 171, 297, 298
Ashby, 54
Atkins, 180
Atwood, 69, 249
Ausemus, 139, 331, 332

B

Barker, 173
Barley, artificial epiphytotics, fusarial head blight, 120
    smuts, 120
    stem rust, 118
  breeding, 88–91
  chromosome numbers, 155
  cross-pollination, 42
  crossing methods, 63
  effects of selfing, 99–100
  inheritance, internode length, 155
    quantitative characters, 163
    reaction to *Helminthosporium sativum*, 159
    reaction to mildew, 162
    reaction to stem rust, 160
    in species crosses, 152–153
  linkage groups, 155–156
  species, 152–153
Bartlett, 341
Bateson, 11
Baur, 114
Beadle, 224
Beans, mosaic resistance, 115
Beddows, 249
Biffin, 132, 133
Bindloss, 56
Blakeslee, 34–36
Blanchard, 241
Bliss, 375, 421
Blodgett, 41
Bolley, 114, 172
Borgeson, 268
Boyack, 132, 133
Bressman, 218
Brewbaker, 194, 247, 301
Brieger, 251
Brierley, 35
Briggs, 104, 139, 140, 162
Brink, 255
Broadfoot, 171
Brookins, 89, 117, 127, 162
Brunson, 189, 197, 236, 239
Bryan, 209, 237, 238, 304
Burlison, 239
Burnham, 110, 173, 224, 235
Bushnell, 250

C

Cabbage, wilt resistance, 115
  yellows, 126
Campbell, 182
Cantaloupe, resistance to mildew, 104
Cartledge, 110, 235
Cartter, 85
Chang, 178, 179
Christidis, 303
Chromosome numbers, in cereals, 12

Chromosome numbers, in fiber plants, 13
in forage grasses, 12–13
in fruits, 14
in legumes, 13
in oats, 141
in oil plants, 13
in stimulants, 13
in sugar plants, 13
in vegetables, 14
Churchward, 139
Clark, Andrew 376
Clark, E. R., 181
Clark, J. A., 83, 131, 132, 139
Clarke, 247
Clayton, 85, 182
Cochran, 292, 352, 367, 375
Coffman, 143, 150
Collins, G. N., 52, 241
Collins, J. L., 40
Combining ability, in corn, 236-239
in small grains, 98
Cooper, D. C., 255
Cooper, H. P., 134
Corn, artificial epiphytotics, smuts, 121, 122
breeding improved inbred lines, by backcross method, 210
by convergent improvement, 52–53, 210–214
by pedigree method, 207
breeding methods, 6–8, 187–214
combining ability, 236–239
controlled pollination methods, 193–214
drought resistance, 181
effects of selfing, 48–50
inheritance, of chlorophyll variations, 221
of endosperm characters, 218
of glossy seedlings, 223
of plant colors, 222
linkage map, 225
linkage studies, 224–231, 233–236
origin and classification, 215–218
pericarp tenderness, 108, 240
protein content, 239
quantitative inheritance, 231
Corn, resistance of, to bacterial wilt, 241
to cold, 239
to drought, 239
to insects, 241
to rust, 241
to smut, 109, 234
seed increase of inbreds and F_1 crosses, 268–269
selection without controlled pollination, 187–191
selfing and crossing technics, 61
Cotton, effects of self-pollination, 47
selfing and crossing, 64
Cox, 352, 367
Craig, 81, 145, 148
Craigie, 136
Crane, 251
Cummings, 48, 250
Curtis, 250
Cutler, 176
Cytogenetics, allopolyploids, 16
autopolyploids, 16

D

Dahms, 241
Darlington, 130
Darwin, 242
Davis, 236
Derman, 34
Dickson, 135, 148
Dillman, 85, 165, 167, 169
Disease resistance, in barley, 90
in beans, 115
in cabbage, 115
types of, 126
in cantaloupes, powdery mildew, 104
in corn, smut, 109
in flax, wilt, 114
in grapes, 114
importance of, 113–116
methods of breeding for, 122–124
nursery methods, 82
in oats, crown rust, 27, 149
smuts, 29, 150
stem rust, 28, 148

Disease resistance, production of epiphytotics, 118–122
reaction to bacterial wilt of corn, 241
reaction to *Helminthosporium sativum*, 159
reaction to insect attacks, 241
reaction to rust of corn, 240
reaction to smut of corn, 234
in snapdragons, rust resistance, 106
in tomatoes, 115
in vegetables, 115
in watermelons, 115
in wheat, bunt, 104
Hessian fly, 127
leaf rust, 107
mature-plant resistance, 4, 117, 127
physiological resistance, 4, 117, 126
stem rust, 107
Doxtator, 202

E

East, 45, 49–51, 53, 77, 193, 218, 221, 232, 239, 242, 251, 254
Eckhardt, 209, 237, 352, 367
Edgerton, 115
Ellerton, 131
Emerson, 219, 222, 224, 231, 232
Emsweller, 35, 66, 106, 251
Engledow, 130, 133, 152

F

Farrer, 87, 139
Fernow, 41
Field plot methods, Latin squares, 312–313
lattice, simple, 351–365
triple, 365–375
multiple experiments, 339–350
randomized blocks, 307–312
replication, 304
size and shape of plots, 302
split-plot designs, 315–320
yield trials in Minnesota, 304–306
Field plot technic, competition, 297
crop rotation, 289–291
soil heterogeneity, 291
Fisher, 287, 295, 307, 308, 312, 322, 331, 375, 411, 417, 418
Flax, artificial epiphytotic, rust, 120
wilt, 120
chromosome numbers, 165
crossing methods, 64
dehiscence of bolls, 168
inflorescence, 166
inheritance, of flower and seed color, 167
of quality of oil, 170
of resistance to rust, 174
of resistance to wilt, 171
smooth vs. ciliate septa, 169
of weight of seed and oil content, 169
of wilt resistance, 114, 171
Flor, 174
Fraps, 219
Fraser, 143, 145, 224
Freeman, 133

G

Gaines, 2, 20, 134, 139, 147
Garber, 39, 60, 147, 157, 158, 218, 236, 239, 293, 296
Garl, 70
Garner, 85
Garrison, 189, 190
Genetics, application of, to plant breeding, 25–34
of barley, 152–164
of bunt resistance, 140
of flax, 165–174
genomes in wheat, 20–21
of maize, 215–241
of oats, 141–151
of plant pathogens, 125–126
of self-incompatibility, 251–257
of wheat, 129–140
Gilman, 115
Glossary, 381–389
Goulden, 81, 136, 139, 351, 363
Grapes, resistance of, to vine louse and vine mildew, 114

Grasses, effects of selfing, 249
selfing methods, 71
Griffee, 89, 160, 161

H

Haber, 239, 250
Hall, A. D., 281
Hall, D. M., 180
Hamilton, 247
Harlan, 84, 88, 97, 98, 101, 152, 153, 155, 157
Harrington, 98, 99, 136, 179, 180
Harris, 292, 293, 296, 320
Harvey, 240
Hauge, 219
Hayes, 5, 23, 39, 49–51, 54, 60, 88, 89, 108, 135, 138, 143, 144, 145, 150–153, 157, 158, 188, 193, 199, 207, 208, 218, 221, 235, 236, 239, 240, 268, 293, 297, 298, 308, 323, 339
Hays, 76
Henkemeyer, 133
Henry, 174
Heribert-Nilsson, 247
Heterosis, explanation of, 50–56
Heyne, 181, 239
Hines, 89, 160
Hitchcock, 216
Hogg, 185
Holbert, 180, 239
Hollowell, 69
Hoover, 234, 296
Hopkins, 187
Hor, 152
Howards, 131, 132
Humphrey, 47, 65
Humphries, 176
Hunter, H., 57
Hunter, J. W., 181
Huskins, 24
Hutchins, 250

I

Immer, 99, 155, 234, 292, 299, 301, 308, 322, 339
Ivanoff, 241

J

Jagger, 250
Jenkin, 71, 73, 247, 249
Jenkins, E. W., 48, 250
Jenkins, M. T., 61, 195, 197, 201, 202, 206, 236, 237, 239
Jodon, 65
Johannsen, 76
Johnson, I. J., 54, 108, 169, 199, 202, 207, 208, 236, 239, 240
Johnson, T., 135, 138
Jones, D. F., 42, 45, 46, 49, 51, 52, 193, 206, 218, 236, 239, 242
Jones, H. A., 66, 104, 106
Jones, J. W., 85
Jones, L. R., 115
Jorgenson, 194

K

Kearney, 47
Keeble, 50
Kempton, 240, 241
Kerns, 40
Kezer, 132, 133
Kiesselbach, 55, 187, 195, 298–300, 302
Kihara, 20, 141
Kirk, 69, 71, 244, 249
Knowles, 179, 180
Kostoff, 20
Kouznetsov, 134
Kurtzweil, 138

L

Larmour, 182
Larson, 179
Latin squares, 312–313
Laude, 181
Lawrence, 251
Leake, 57
Leith, 89
Leonard, 153, 376
Levy, 259
Lilienfeld, 20
Lindstrom, 55, 233

Link, 185
Love, H. H., 81, 145, 147, 148
Love, R. M., 23
Luckwill, 55

M

Macauley, 259
McFadden, 6, 22, 138
McIllvaine, 296
Macindoe, 137, 138
McRostie, 147
MacVicar, 185
Mains, 240
Mangelsdorf, 215, 216, 219, 221
Marston, 241
Martini, 84, 98
Mayer, 194
Mendel, 11, 86
Mercer, 281
Meyers, 241
Missing plots, estimating yield of, 314
Montgomery, 187
Moore, 23, 143, 144, 323
Morse, 85
Muller, 2
Murphy, H. C., 150
Murphy, R. P., 213
Mutations, induced in wheat, oats, and barley, 21
  nondefective, 78
Myers, 22, 169, 174, 241

N

Neal, 195
Neatby, 136, 139
Nebel, 36
Newman, 75
Newton, 135, 138
Nilsson, H. N., 75
Nilsson-Ehle, 133, 134, 145, 148
Nilsson-Leissner, 134, 194
Nishiyama, 141, 143
Noll, 81
Nowosad, 185

O

Oats, artificial epiphytotics, crown rust, 118
    smuts, 120
    stem rust, 118
  breeding of, 25–34, 92
  crossing methods, 63
  fatuoids, 24
  inflorescence, 146
  inheritance, of awn development, 144
    of byzantina-sativa characters, 23, 30–33
    of color of grain, 145
    of crown rust reaction, 27, 149
    of hulled vs. hull-less, 147
    of pubescence, 148
    of quantitative characters, 151
    of smut reaction, 29, 150
    of spreading vs. side panicle, 147
    of stem rust reaction, 28, 148
  loose smut, 124
  quantitative characters, 33, 151
  species crosses, 141–144
  stem rust, 28, 117, 123
Onion, selfing and crossing, 66

P

Painter, 127
Pan, 139
Parker, J. H., 138, 143
Parker, W. H., 134
Pearl, 3
Pearson, Karl, 283
Pearson, O. H., 258
Pellew, 50
Pelshenke, 176
Percival, 132
Peterson, 138, 247
Philp, 24, 143, 147, 148
Physiologic races, Hessian fly, 122
  loose smut of oats, 124
  rust in flax, 174
  stem rust, of oats, 123
    of wheat, 4–6, 123, 135
  wilt of flax, 171

Pieters, 69
Plant breeding, applications of genetics, 25–34
colchicine in, 34–38
hybridization methods in self-pollinated plants, 86–100
outline of procedure, 91–98
important phases, 1
methods, asexual groups, 39–41
backcross, 97, 101–112
bulk, 96
classification, 56
convergent improvement, 52–53
corn breeding, 6–8, 187–214
cross-pollinated plants, 257–262
disease and insect resistance, 113–128
multiple crosses, 97
pedigree, 95
potato improvement, 8–10
pure line, 74–85
outline of procedure, 79–83
in Scandinavia, 75
sexual group, 41–50
technics in selfing and crossing, 60–73
value of, 2
Vilmorin's isolation principle, 75
polyploids in relation to, 15–25
selection methods, for cold resistance, 177
for coumarin content in sweet clover, 182
for dormancy in wheat, 179
for drought resistance in corn, 181
for HCN in sudan grass, 185
for lodging resistance in small grains and corn, 180
for quality in wheat, 175
for shattering resistance in wheat, 178
for wheat-meal fermentation time test, 176
of wheat, rust resistance, 3–6
Polyploids, autopolyploid inheritance, 18–19
Polyploids, origin of, 16
in relation to plant breeding, 15–25
Pomeroy, 40
Poole, 250
Pope, 101
Porter, 250
Potatoes, breeding methods, 8–10
crossing methods, 66
Powers, 22, 23, 43, 89, 135, 160, 163, 164, 308, 339
Prell, 251
Pumpkin, selfing and crossing, 66
Punnett, 11

Q

Quinby, 65
Quisenberry, 131, 132, 143, 177, 236

R

Raleigh, 292
Randomized blocks, 307–312
multiple experiments, 339–350
Rasmussen, 164
Red clover, effects of selfing, 249
selfing and crossing methods, 67
Reed, 124, 125, 148, 150
Reeves, 215, 216
Reid, 89, 161
Rice, crossing methods, 65
Richey, 52, 53, 102, 189–191, 194, 195, 197, 210
Rider, 332
Rieman, 115
Riker, 241
Riley, 253, 254
Roberts, 185
Robertson, 152, 153, 155
Rosa, 250
Ruttle, 36
Rye, effects of selfing, 247
selfing and crossing methods, 64

S

Saboe, 235
Salmon, 180, 376

Sansome, 24
Saunders, 176
Scofield, 296
Scott, G. W., 250
Scott, L. B., 39
Seed certification, 263–279
  Canadian Seed Growers' Association, 265, 269
  increase by Minnesota method, 266
  International Crop Improvement Association, 270
  Minnesota method, 272–277
  potatoes, 277
    Maine method, 278–279
  recommended varieties, 264
  selecting the variety, 263
Self-fertilization, effects of, in alfalfa, 244–247
  in corn, 48, 242
  in cotton, 47
  in cross-pollinated plants, 48–50
  formulas, 45–46
  in often cross-pollinated plants, 46–47
  in rye, 247
  in sunflowers, 247
  in timothy, 247
Shamel, 39, 40
Shaw, 168
Shull, 50, 192, 193
Singleton, 206
Smith, D. C., 117, 123, 127, 148, 150
Smith, H. H., 36
Smith, L. H., 189
Snapdragons, rust resistance, 106
  tetraploid, 35
Snedecor, 310, 335, 375, 412, 419
Snelling, 241
Sorghum, artificial epiphytotics, smut, 121–122
  crossing methods, 65
Spillman, 134
Sprague, 52, 53, 55, 210, 238
Squash, selfing and crossing methods, 66
Stadler, 21
Stakman, 23, 125, 126, 135, 138, 143, 144, 323
Stanford, 162
Stanton, 84, 92, 141, 150
Statistics, applied to data in percentages, 375–379
  Chi square tests, 320–324
  comparison of varieties through check, 350–351
  correlation, multiple, 338
    partial, 333–338
    simple, 284, 325–329
      means and differences of, 331–333
  definition of constants, 280–281
  mean, mode, standard error, variance, coefficient of variation, 281–284
  regression, 329–331
  t test, 285–288
  tables of F, 412–416
  tables of $p = \sin^2 \theta$, 421–423
  tables of r and R, 419–420
  tables of r to z, 418
  tables of t, 411
  tables of χ^2, 417
Stephens, 65
Stevenson, F. J., 42
Stevenson, T. M., 69, 182, 185
Sturtevant, 217
Summerby, 297
Suneson, 64
Sunflowers, effects of selfing, 247
Surface, 143, 145, 148
Sweet clover, coumarin content, 182
  inducing biennial habit, 182
  selfing and crossing methods, 69

T

Tammes, 165, 167
Tedin, 307
Timothy, selfing effects, 247
Tingey, 92
Tippett, 308
Tisdale, 171
Tomatoes, cross-pollination, 42
  wilt resistance, 115

Torrie, 143, 144, 147, 150
Trost, 219
Tuller, 65
Tysdal, 70, 244, 246, 299

V

Valle, 247
Vavilov, 34, 88, 134, 215
Vegetables, disease resistance, 115
Vilmorin, 75
Vogel, 178

W

Wade, 85
Walker, 126
Wallace, 218, 335
Wang, 56
Warmke, 35
Watermelons, wilt resistance, 115
Watkins, 129, 131
Weibel, 177
Weihing, 302
Wellhausen, 241
Welsh, 136, 139, 148, 149
Welton, 189
Wexelsen, 159
Whaley, 56
Wheat, artificial epiphytotics, bunt, 119
  fusarial head blight, 120
  Hessian fly, 122
  leaf rust, 118
  stem rust, 118
  bunt resistance, 104
  crossing methods, 63
Wheat, cross-pollination, 43
  genomes, 20–21, 129
  germinal instability, 22–23
  Hope and H44, 6, 23
  inflorescence, 62
  inheritance, of awnedness, 130
    of bunt resistance, 139
    of chaff characters, 132
    of glume shape, 129
    of quantitative characters, 140
    of seed characters, 133
    of spike density, 134
    of spring vs. winter habit, 134
    of stem rust reaction, 135
  kernel color, 17
  leaf rust, 107
  resistance to Hessian fly, 127
  speltoids, 24
  Thatcher, 5–6
Whitaker, 250
White, 185
Wiebe, 155, 352, 356, 365
Wiener, 269
Williams, C. G., 187, 189
Williams, R. P., 67, 249
Wilson, 181
Winge, 19, 24
Woodward, 92
Worzella, 176
Wright, 45
Wu, 208, 236

Y

Yasuda, 255
Yates, 308, 312, 314, 351, 352, 362, 375