GENETIC AND CERTAIN BIOCHEMICAL CHARACTERISATION STUDIES OF SOME SCENTED VARIETIES OF RICE

B. P. S. CHAUHAN
M.Sc. (Genetics)

MARCH 1981

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Thesis approved for the award of the
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Osmania University.

Controller of Examinations

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M. Sc. (Genetics)

THESIS SUBMITTED FOR
THE DEGREE OF
DOCTOR OF PHILOSOPHY
OSMANIA UNIVERSITY

MARCH 1981

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C E R T I F I C A T E

This is to certify that the thesis entitled " GENETIC AND CERTAIN BIOCHEMICAL CHARACTERISATION STUDIES OF SOME SCENTED VARIETIES OF RICE " submitted for the Degree of DOCTOR OF PHILOSOPHY in Genetics, Osmania University, is a record of bona fide research carried out by Mr. B.P.S. Shekhar, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help taken during the course of investigation and the source of literature have been fully acknowledged.

Date: March 11, 1981.

(G.M. REDDY)

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(G.M. REDDY)

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D E D I C A T E D

TO

M Y P A R E N T S

CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	6
MATERIALS AND METHODS	26
OBSERVATIONS	50
I. Genetic Studies . ..	50
II. Esterase Isoenzyme studies	53
III. Lipoxygenase enzyme studies	62
IV. Volatile flavor component studies.	65
V. Lipids and fatty acid composition studies. ..	70
VI. Amino acid composition studies	73
DISCUSSION	78
SUMMARY	104
REFERENCES	i - ix

LIST OF TABLES

S.No.	Title	Page Nos.
1.	Varieties used for genetic studies	26
2.	Varieties used for Esterase isoenzyme studies.	29 '& 30
3.	Aroma pattern in F_1 and F_2 populations of different croses.	52
4.	Presence of esterase isoenzyme bands in different rice varieties.	55 & 56
5.	Esterase activity (Relative percentage) in different varieties of rice.	57 & 58
6.	Specific activity of lipoxygenase in different rice varieties.	64
7.	Mean values of volatile in different rice varieties.	66
8.	Comparision of mean volatile values among scented and non-scented varieties.	67
9.	Comparision of volatile among high, medium and low scented rice varieties.	68
10.	Fatty acid composition of different rice varieties.	72
11.	Amino acid composition of different rice varieties.	74
12.	Comparision of lipoxygenase activity and linoleic acid content in different rice varieties.	91
13.	Strecker's degredation of amino acids.	After 101

LIST OF FIGURES

S.No.	Title	After page
1.	(a,b,c) Esterase Isoenzyme pattern in different rice varieties.	59
2.	(a,b,c,d,e,f) Gel scans along with diagramatic representation of banding pattern in different rice varieties.	60
3.	(a,b,c,d) Variation in lipoxygenase activity as a function of pH and time in different rice varieties.	62
4.	Specific activity of lipoxygenase enzyme in different rice varieties.	64
5.	Profiles of volatiles in some rice varieties.	68
6.	Gas chromatogram of the aroma concentrate with flavor comments. Var. Randhunipagalu.	69
7.	Gas chromatogram of the aroma concentrate var. Randhunipagalu.	69
8.	Gas chromatogram of the aroma concentrate var. Godavari Isuka.	69
9.	Gas chromatogram of the aroma concentrate var. Type 3.	69
10.	Gas chromatogram of the aroma concentrate var. Basumati 370.	69
11.	Gas chromatogram of the aroma concentrate var. Jaya.	69
12.	a and b. TLC separation of lipid from different rice varieties.	70
13.	Typical separation of fatty acid methyl esters.	71
14.	Proline and alanine composition in different rice varieties.	75
15.	Essential amino acid profiles in different rice varieties.	76
16.	Scheme of oxidation of linoleic acid.	91

INTRODUCTION

Rice is a staple food of the tropics and accounts for more than 21% of the total food calories consumed by the World's population (Athwal 1971). Consumer preferences were mainly responsible in selecting varieties, with high amylose and soft gel consistency which are preferred in India, with intermediate amylose which are preferred in Oceania and low-amylose rices which are preferred in Japan and South Korea. Besides these, some varieties show extreme elongation qualities such as Sadri varieties from Iran and some varieties from Burma (Juliano 1979). Scented rices occupy a unique place in the sphere of consumer preference due to their pleasant aroma and desirable elongation qualities upon cooking (Choudhury and Ghosh 1978). They are being traditionally cultivated in the Indian sub-continent since ancient days and are popularly referred to as 'Basmati rices'.

Basmati rices command a pre-eminent place in the World market, in view of the attractive price and have great export value. Apart from these features, high milling returns (Anonymous 1972), tolerance and resistance to certain pests (Reddy 1964) are also associated

with these rices. Considering these attributes, breeding for high yielding scented varieties has assumed National importance. Genetic and biochemical studies, especially active principles of scented rices have not been fully understood, and the need for these investigations has been stressed by many research workers (Sood 1978, Choudhury and Ghosh 1978 and Anonymous 1980).

In view of the growing importance of basmati rices, the present investigation was undertaken and specific studies with Esterase isoenzyme and lipoxygenase enzyme in scented and non-scented varieties, characterisation of volatile flavor components, lipids, fatty acid and amino acid composition etc., were carried.

The genetics of 'aroma' was studied among the segregating material of five crosses involving scented and non-scented varieties. The F_1 plants were non-scented and F_2 data fitted in monogenic ratio of 3:1 (non-scented : scented), suggesting that 'aroma' in rice is governed by a single recessive gene.

Esterase isoenzyme pattern in five day old seedlings of thirty scented and six non-scented rice varie-

ties suggest that non-scented varieties exhibit two specific bands, band 8 (Rf 0.41) and band 12(Rf 0.63), which were absent in all the scented varieties. Among the scented varieties also, there were differences in the electrophoretic pattern and they could be grouped into a four categories, based on the gradation of aroma and zymogram pattern.

Lipoxygenase enzyme was studied quantitatively in scented and non-scented varieties and the scented varieties had lower values than the non-scented ones. This shows that these varieties have better storage qualities, since this enzyme is mainly responsible for the formation of off-flavors during storage.

Volatile flavor components were studied in different scented and non-scented varieties both quantitatively and by Gas Liquid Chromatography. All the scented varieties had significantly higher amounts of volatiles than the non-scented. Among scented varieties, there were significant differences between the varieties with known gradation of scent such as high, medium and low scented varieties. These observations suggest that scented varieties are characterised by

the presence of higher amounts of volatiles. The GLC chromatograms of scented and non-scented varieties, showed 17 to 19 peaks and there were differences in the compositional pattern among different varieties. Scented varieties had prominent peaks in the initial stages of the chromatograms and all the scented varieties had a large peak 6, which was insignificant in non-scented varieties.

Lipids and fatty acid composition was studied in twenty scented and two non-scented varieties. TLC of lipids has showed that there were no major class differences among the varieties. Fatty acid composition by GLC showed that eight fatty acids were present in all the varieties, and the scented varieties had oleic acid in higher quantities suggesting that this may be associated in the biosynthesis of flavor compounds.

Amino acid composition studies of different scented and non-scented varieties have shown a wide variation. Proline and alanine, which are precursors for certain volatile flavor compounds, were present in higher quantities in scented varieties than in non-scented varieties. The essential amino acid pattern

was compared among the varieties with that of FAO recommended values. For most of the essential amino acids, all the scented varieties had comparable values to that of the recommended values and this shows that scented rice varieties have better amino acid composition.

The present studies clearly suggest that scented rices can be characterised biochemically, by enzymes like esterase, lipoxygenase, by quantitative differences in fatty acid and amino acid composition and volatile flavor components. The simple monogenic inheritance pattern of scentedness, besides the above biochemical parameters can be exploited in utilising this economically important character in breeding for better yield.

REVIEW OF LITERATURE

Distribution of scented rices:

Scented (Basmati) rices are being cultivated in Indian sub-continent beside other countries like Japan, Indonesia etc. since ancient times. In Japan the scented rice varieties are referred to as "Kabashika", "Niomia", "Jakomai" etc., and are usually served on festive occassions (Tsuzuki et al 1975 a).

In our country basmati rices are mainly grown in the Northern India comprising Punjab, Haryana, Uttar Pradesh, Rajasthan and Delhi and to a certain extent in states like Andhra Pradesh, West Bengal, Karnataka, Maharashtra etc., (Siddiq et al 1976).

Richharia et al (1965) in their studies on the World Rice genetic stocks, studied the distribution of scented rice varieties and found that the percentage of these varieties is higher in temperate zone than in other zones. However in all the zones the total percentage was low and they concluded that the origin of these rices is very recent, and the character has been retained in the temperate zone than in other rice growing regions. They also found that most of the varie-

ties fell in the medium duration group, less in the late duration group and least in the early duration group. Scented varieties are not found in the wild species of the genus Oryza and are confined to only O. sativa. species.

In cereals, scented sorghums have been reported in a collection from Madhya Pradesh (Rao and Murty 1979), and emit similar type of "aroma" as that of basmati rices when treated with mild alkali solution.

Genetics of Scented rices:

Ramaiah (1937), Dave (1940) and Srinivasan(1941) reported that scent is monogenic and dominant to non-scentedness. A digenic segregation of 9:7 (scented : non-scented) was observed by Ramaiah (1937), while a ratio of 15:1 (scented : non-scented) was reported by Srinivasan (1941).

Kadam and Patankar (1938) found a trigenic ratio of 27:37 (scented : non-scented). Dave (1940), found xenia effect for this character obtaining segregation in the F_1 hybrid. Chakrabarthy (1948) has reported an F_2 segregation of 13:3 (non-scented : scented) and

interpreted the phenomenon as due to inhibitory factors. Ghose and Butany (1952) have found non-scented trait to be monogenic dominant to scented. The three complimentary genes have been designated as O_a, O_b and O_c, while the duplicate genes as O_1 and O_2, by Ramaiah (1953 a).

The inheritance pattern of scent was identified generally by breaking the rice grains under the teeth or by boiling the grains. Ramaiah and Rao (1953), pointed out that these diverse genetic ratios were obtained owing to the difficulties in identifying and scoring scent.

Nagaraj et al (1975) studied inheritance of scent on the basis of leaf analysis i.e., identification of scent from leaves, and they found a ratio of 27 scented : 37 non-scented. This indicates that scent is governed by the simultaneous action of three dominant genes. Dhulappanavar (1976), studied the inheritance pattern of scent and its association with pigmented characters and found four complimentary genes governing scent. One of the gene showed linkage with a complimentary gene for red pigment in apiculus, which is part of V linkage group.

Reddy (1977) recovered a non-scented mutant from the scented cultivar HR-47 and found that it segregated for scented and non-scented plants in M_3 generation. It was suggested that the loss of scent is due to a recessive mutation. In a recent study, Sood and Siddiq (1978) found that the scent trait is monogenic recessive and segregates in a ratio of 3 non-scented: 1 scented in the F_2 generation. Their identification of scent was based on the treatment of plant material to mild alkali solution. In their observations the F_1 plant was non-scented in all the cross combinations.

Mutational Studies:

Reddy and Reddy (1971) obtained promising dwarf mutants from a tall local scented variety, HR-47, which matured earlier and yielded 17% more when compared to control. In a similar way dwarf mutants have been induced in scented varieties like NP 49, NP 114 and T 412. Apart from being dwarf, they had resistance to the principal rice diseases like, blast, bacterial leaf blight and tungro virus (Misra as quoted by Gangadharan et al 1976).

Gangadharan et al (1976) induced mutations in the scented variety Basmati-370 by treating it with

gamma rays. In M_4 generation 16 semidwarfs were isolated and they bred true in M_5. These mutants were shorter in height, had more ear bearing tillers and gave higher yields (33% - 133% more). Some of the mutants had better kernel elongation than the control. Chaudhary et al (1979), isolated early maturing and dwarf mutants from six scented rice varieties of Uttar Pradesh. Mutants of some varieties out yielded the parents in multi location tests and it was concluded that mutation breeding was effective in inducing productive dwarfs in scented rices.

Physico-chemical Studies:

Scented rices have special features in their grain and cooking qualities. Juliano, (1966) has shown that, scented rices are characterized by having amylose content around 20% and gelatinisation temperature in the range of medium to low. This was an interesting revelation since these characteristics are distinctly different from those of the typical indica rices. Raghavayya and Kaul (1970), in a study with scented and non-scented rice varieties, found that the scented varieties had a higher fat content and also

higher ash content. The varieties also had better elongation after cooking when compared to ordinary varieties. Protein percentage was also high in some varieties (8 to 10%).

Sood (1978), studied the amylose content, protein content, gelatinisation temperature, volume expansion, water uptake, L/B ratios of a number of scented varieties from different geographical regions. The study showed that amylose percentage was around 20% in most of the varieties and it varied from 14% to 25%. Protein content varied from 9% to 12%.

Choudhary and Ghosh (1978), in their study on the agronomic and physico-chemical properties of scented and fine rices, found that some of the scented rices like 'Seetabhog', 'Badshabhog' etc., gave higher yields and greater kernel elongation ratio than typical 'Basmati' types. They observed more linear elongation on cooking not only in long-grained 'Basmati' types but also in short-grained scented rices like 'NC 314' and 'Kalanamak'. Juliano (1979), in a recent review on the quality of rice, indicated that Basmati rices have lower birefringence end-point temperature (BEPT) and intermediate amylose content. They have extreme

elongation qualities, and although other varieties also had similar combinations of BEPT and amylose content, they do not show the same elongation. All the scented varieties showed medium gel consistency.

Esterase Enzyme Studies:

Esterase enzyme splits simple esters in biological systems (Cherry and Crandall 1932) and has been shown to exist in multiple forms both in animals (Augustinnson 1961) as well as in plants (Qubadda and Quattrucci 1974). Esterase isoenzymes have been studied in the members of the family Fabaceae (Thurman et al 1967), Triticinae (Barber et al 1968 and Bhatia 1968), in Brassica species (Vaughon 1967) and in Hordeum species (Mitra et al 1970) for the evaluation of phylogenetic and evolutionary trends.

By using gel electrofocussing technique, Qubadda and Quattrucci (1974) studied the esterase isoenzymes in wheat and by adopting substrate and inhibitor specificity, it was shown that the enzymes are members of aliesterases or esterase B (3.1.1.1).

Payne and Koszykowski (1978) studied the esterase isoenzyme differences among 44 soybean cultivars, with

a view to identify varieties based on isoenzyme differences. They concluded that these studies can be useful in a testing programme to identify soybean cultivars, since the quantity of extract from a single soybean seed is sufficient for the analyses.

Esterase isoenzymes have been well studied in relation to the origin and inter-relationship among the genus Oryza. Nakagahra and Hayashi (1977), studied esterase isoenzyme pattern in more than 1,000 varieties of rice from different regions of the World. From these studies, they concluded that the center of genetic diversity for rice exists in areas like East Nepal, Bhutan, Assam etc.

Palanichamy and Siddiq (1977) studied inter-relationship among A genome species of rice and concluded that the entire A genome species could be grouped into "sativa" and "glaberrima" complexes.

Lipoxygenase Enzymes Studies:

Lipoxygenase (linoleate : oxygen oxido-reductase E.C. 1.13.1.13) is found in a wide variety of plants, particularly in legumes (Tappel 1963). It is implicated with heme proteins and specifically catalyzes

the oxidation of methylene-interrupted unsaturated fatty acids such as linoleic, linolenic and arachidonic acids and their esters to their respective peroxides (Holman and Bergstorm 1951). This enzyme leads to the deterioration of fat-soluble vitamins and fatty acids of oils and fats. It also produces off flavors and odors because of its action on unsaturated fatty acids in the lipids of food materials (Shastry and Rao, 1975).

Lipoxygenase has been reported in a number of plants. It is present in alfalfa (Ben-Aziz et al 1971), groundnut (Dillard et al 1960), soy-beans (Balls et al 1943), Peas (Siddiqui and Tappel 1956 and Erikson and Svenson 1970), Potato (Pinsky et al 1973), and Wheat (Irvine and Anderson 1953). Lipoxygenase from soy-beans has been well studied and also been characterised (Holman 1947, Koch et al 1958 and Garsson et al 1972).

Guss et al (1967) in their study of isozymes of lipoxygenase, reported four bands in soybean, one in mung bean and four in heat. Christopher et al (1970 and 1972) obtained two isoenzymes from soybean after elution with DEAE - Sephadex.

In rice, studies on this enzyme have been rather limited. Shastry and Rao (1975), studied lipoxygenase from rice bran and they found that the optinum pH for this enzyme was about 8.5. They also carried disc-gel electrophoresis and found three distinct bands with lipoxygenase activity. They reported that, tryptophan specific reagents had a considerable inhibitory effect on the partially purified enzyme and concluded that the rice enzyme may require tryptophan residues for its activity.

Yamamoto et al (1980), studied lipoxygenase from rice bran and germ of different 'japonica' rice varieties. They found that most of the activity was localised in the germ portion. They purified the enzyme by ammonium sulphate precipitation, heat treatment and column chromatography on DEAE-Sephadex and estimated the molecular weight to be 95,000. It was found that the isolated enzyme had typical properties of lipoxygenase, which were comparable to soybean lipoxidase. The optimal pH was around 6.5 to 7.0 and the discrepancies with the results of Shastry and Rao (1975), were explained as to the variety and tissue variation of the enzyme and 'japonica' versus 'indica' varieties.

Flavor components studies:

Although great deal of work has been done with respect to the physico-chemical properties of rice grain, not much is known about the flavor components of rice. Obata and Tanaka (1965), identified hydrogen sulfide, ammonia and carbon dioxide in the vapors of cooked rice and Yasumatsu et al (1966), found volatile carbonyl compounds such as acetaldehyde, propanal, 2-butanone, pentanal and discussed their formation.

Kurasawa et al (1969), in their study on the flavor components of rice cake, observed that the flavor components were extractable with hot water. They identified hydrogen sulfide, ammonia, carbon dioxide and some carbonyl compounds. It was concluded that no single compound was responsible for the flavor.

Bullard and Holguin (1977), studied the volatile flavor components of raw rice, with respect to the attractiveness of rats to rice fields and identified 73 compounds, of which only 9 were previously reported in rice. The compounds included alcohols, aldehydes, alkyl aromatics, furans, ketones, terpenes and naphthalenes. Legendre et al (1978) studied the volatiles

from rice and corn products and found methanol, acetaldehyde, ethanol, acetone, pentanal, hexanal and hexanol in volatiles of whole rice, brown rice and polished rice. In addition to these, 2 methylpropanol, 2 methyl pyrazine, furfural, 2-5 dimethyl-pyrazine, benzaldehyde and 2,6-dibutyl-4-methyl-phenol were found in breakfast cereals of rice.

Yazima et al (1978), in their study of volatile flavor components of cooked rice, found 100 constituents of the flavor, including 13 hydrocarbons, 13 alcohols, 16 aldehydes, 14 ketones, 14 acids, 8 esters, 5 phenols, 3 pyridines, 6 pyrazines and 8 other compounds. Of these 100 components, 92 were newly identified volatile flavor components of cooked rice.

Tsugita et al (1980), studied the volatiles of cooked rice, which was milled to different degrees (92, 85, 75 and 50% milled rice) by GC and GC-MS techniques. Forty volatile compounds were identified in cooked rice and there were marked differences in both the odor of cooked rice and in the quantity of volatile components between 92% milled rice and the other degrees of milling. They also evaluated the aroma concentrates organo-leptically and concluded that the

surface layer constituents of the rice grain have an important role in the formation of the odor of cooked rice.

Mitsuda et al (1968), analysed the volatiles from rice bran, and identified several compounds like methanol, ethanol, n-hexanol, acetaldehyde, acetone, methylpropanol, pentanal and hexanal. Fujimaki et al (1977), studied the volatiles from the steam distillate of rice bran, and found that the neutral fraction was the major fraction and was indespensable for producing the rice bran odor. They found 17 carboxylic acids and 7 phenols. 4-Vinyl phenol was the main component in the steam distillate of rice bran, having a characteristic unpleasant odor.

Tsugita et al (1978), analysed the neutral and basic compounds from the steam distillate of rice bran. They found a total of 146 compounds (alkanes, aromatic hydrocarbons, alcohols, aldehydes, ketones, esters, lactones, furans, pyridines, pyrazines, quinolines and thiazoles etc.) in addition to the 17 acids and 7 phenols. 2 acetylthiazole and benzothiazole were considered to be the key compounds of the rice bran odor and other compounds like lactones were also found

to be necessary for reproduction of the odor.

As regards the studies on flavor components of scented rice, Ayano and Furuhashi (1970) were the first to make an attempt in this direction. They reported an increase in 2-pentanone content by adding scented rice to ordinary rice during cooking.

Tsuzuki et al (1975 a and b, 1977 and 1978) in a series of experiments, reported the volatile flavor components of scented rices. They analysed the head space evolved during cooking scented and normal rices, by gas chromatography. They recognised 19 peaks and found that if the cooking time was longer, more flavor components were apt to occur. But no qualitative differences were found in scented and normal varieties (1975 a).

They studied the volatile carbonyl compounds, evolved during cooking of rice by means of thin layer and gas chromatographies. The volatile carbonyls were trapped as 2,4 dinitrophenylhydrazone derivatives. In TLC, 4 spots were recognised and in GLC 19 peaks were identified (1975 b). These workers studied 42 scented varieties grown in Japan, with respect to their diffe-

rences in flavor components. They found varietal differences among the cultivars and they were divided into 8 groups based on their gas chromatograms. They also found that scented rices evolved sharper odor than ordinary ones and the degree of flavor differed with different varieties (1977).

They also studied the sulfur-containing volatiles evolved from cooking scented and ordinary varieties. By flame ionisation detector, methyl mercaptan, dimethyl sulfide and dimethyl disulfide were identified and by flame photometric detector, 5 compounds were identified. But no qualitative differences were found between scented and ordinary rice varieties (1978).

Yazima et al (1979) studied the volatile flavor components of cooked japonica scented rice (Kaorimai) and compared with the composition of ordinary rice variety. The extract was separated into acidic, basic and neutral fractions and all the fractions were examined by combined gas chromatography-mass spectrometry. A total of 114 compounds were identified and 1-pyrrolidine was identified for the first time, as a flavor component of cooked rice and was further observed that

scented rice contained more indole than ordinary rice. It also has an unknown compound and 1-pyrrolidine in addition to the compounds of non-scented variety. They concluded that, although the characteristic flavor components were not found, the difference in scented of non-scented rice varieties is due to the difference in the balance or proportions of their various components.

Lipids and Fatty acid composition: studies:

The role of lipids, especially of fatty acids in the developmentof numerous aroma compounds has been very well studied in the case of fruits (Tang and Jennings 1968, Forss 1969 and Tressl and Jennings 1972). Goldstein and Wick (1969), showed a marked change in the fatty acid composition of banana pulp lipid during ripening. Tressl and Drawert (1973) investigated the enzymatic conversion of linoleic and linolenic acids into volatile carbonyl compounds in banana pulp and observed that palmitic, linoleic and linolenic acids increased in concentration before climacteric rise but decreased in the maximum formation of aroma substances.

Gholap and Bandyopadhyay, (1973 and 1975) studied the contribution of lipid to aroma of ripening mango and found that the ripening was associated with a marked increase in glyceride content. Although the same type of fatty acids were present, a variation in the distribution of fatty acids during ripening was observed and the ratio of palmitic to palmitoleic acid was higher during ripening.

Rao and Bandyopadhyay (1978) investigated the changes in fatty acid composition in pulp lipid of banana during ripening. They found that although similar types of fatty acids were present, considerable variation in their quantitative distribution was existing at each stage of ripening.

In rice, studies on the lipid and fatty acid composition have been limited. Lugay and Juliano(1964) studied by GLC, fatty acid composition of 4 rice varieties. They showed that bran lipids had significantly higher mean content of linoleic and linolenic acids, but lower contents of myristic, palmitic, palmitoleic and stearic acids than milled rice lipids. Nine fatty acids were detected in all, the principal ones being, oleic, linoleic and palmitic.

Yasumatsu and Moritaka (1964) studied the influence of variety, soil and climate and storage temperature on the fatty acid composition of two Japanese rice varieties. Lee et al (1965) studied the effect of storage time on the compositional patterns of rice fatty acids. Significant variation occurred from one sampling period to the next, but significant correlation between percentage composition and time was found in only two cases, oleic and linoleic acid.

Resurreccion and Juliano (1975) analysed fatty acid composition of two rice varieties, by using both petroleum ether and chloroform:methanol as extracting solvents. The major fatty acids were palmitic, oleic and linoleic with traces of myristic, stearic, linolenic and palmitoleic. Milled rice extracted with chloroform:methanol contained more linoleic less oleic acid than oil extracted with petroleum ether. It was concluded that the differences in the fatty acid composition may be due to the choice of extracting solvent.

Amino acid composition studies:

Rice protein is the best among cereals as regards its nutritive value is considered, since it has the best aminogram or essential amino acid composition. Though rice has the highest content of lysine compared to other cereals, its first limiting amino acid is lysine, followed by threonine and tryptophan. (Juliano 1972 b). The aminograms of brown rice and its fractions show large variation in different varieties like the variety BPI-76 which has 0.5% lower lysine than other cultivars with the same protein content (Palmiano et al 1968).

As the protein content increases, the lysine content decreases, but the drop in lysine content is less than proportional to the increase in protein content and does not occur above 10 percentage points of protein (Juliano 1972a). However Austin et al (1972) observed than some lines had relatively more lysine combined with protein thus indicating the possibility of developing strains having high protein and high lysine by extensive screening programmes and adopting suitable breeding methods.

In a study of the amino acid composition of rice and rice by-products, Houston et al (1969), found relatively minor variation for amino acid content of milled rice, where methionine showed maximum variability followed by lysine, threonine, histidine and valine, and histidine and lysine were negatively correlated with protein content and methionine, valine and lysine were positively correlated.

Besides their influence on the nutritional quality, amino acids have a role in the formation of volatile flavor components also. Amino acids change to aldelydes of one less carbon atom on heating with sugars, this reaction being referred to as 'Strecker's degradation'. (Chikubu 1970). Pyridine derivatives such as pyridine, 2-methyl pyridine, 3-methyl pyridine, 4-vinyl pyridine etc., are known to be formed by the amino acids in rice and 1-pyrrolidine is the degradation product of proline (Yazima et al 1979).

MATERIALS AND METHODS

An attempt was made to collect the germplasm of scented rices from different institutes like Central Rice Research Institute, Cuttack, Indian Agricultural Research Institute, New Delhi and International Rice Research Institute, Philippines, besides local areas. About 120 varieties were collected, which exhibited varying degrees of scentedness for the present study.

Genetic Studies:

The following varieties were used for genetic studies.

TABLE - 1

Sl. No.	Variety	Plant ht.	Days to mat.	Source
1.	Randhunipagalu (scented)	115.5	145	C.R.R.I.,Cuttack
2.	Type - 3 (scented)	98.0	135	I.A.R.I.,New Delhi
3.	Basmati-370 (scented)	95.0	140	I.A.R.I.,New Delhi
4.	Pokura(scented)	130.0	115	Local collection from Medak Dist.A.P.
5.	TN(1) (non-scented)	95.0	130	Germplasm collection of Plant Genetics Section,Dept.of Genetics,O.U., Hyderabad.
6.	IR-8, (non-scented)	90.0	135	
7.	Deepaiguda (non-scented)	125.0	115	Collection from A.R.S., Adilabad.
8.	Bala (non-scented)	90.0	110	C.R.R.I,Cuttack.

The following crosses were made between scented and non-scented varieties, (1) Randhunipagalu x TN(1), (2) Randhunipagalu x IR8, (3) Pokura x Deepaiguda, (4) Bala x Pokura, (5) Type-3 x Bala and one cross among the scented varieties (6) Basmati 370 x Type-3. The varieties were selected on the nature of aroma such as 'Randhunipagalu' which is highly scented, 'Type-3' which is medium scented, 'Pokura' which is a local scented cultivar. Bala and TN(1) were selected as they have good combining ability.

Crosses were made by hand emasculation and pollination. Maturing panicles were selected and opened spikelets were clipped off. The spikelets which are likely to open next morning were selected for emasculation. The emasculation generally was carried in the evenings. The spikelets were cut with scissors and all the six anthers were carefully removed with the help of a fine forceps. After emasculation the panicles were covered with glassine bags to prevent pollination. Pollination was done during early mornings. Anthers were collected from the pollen parent and they were pressed to release pollen. Pollen was applied to the emasculated spikelets with the help of a fine forceps.

Immediately after pollination, the panicles were covered with glassine bags and labelled. Usually about 10 panicles were pallinated for each cross. The F_1 seed was collected, about 20-25 days after pollination and were preserved. All the crosses were made in the Kharif Season.

Half of the F_1 seed was preserved and the other half was used to raise the F_2 population. The seeds were sown in earthen pots filled with regular black soil. About 20 day old seedlings were transplanted into pots filled with fine puddled soil.

Segregation studies were made in the F_2 population according to the method of Sood and Siddiq (1978). About a month after transplantation, one or two leaves were cut from individual plants and after cutting into pieces, were placed in test tubes containing 10 ml. of 1.7% KOH (w/v) solution. The tubes were immediately stoppered and the analysis of the tubes was carried after 5 minutes. Scented plants give a typical characteristic 'aroma' as compared to non-scented plants. By the same method, the F_1 plants were also scored and the observations were tabulated. The goodness of fit of the observed frequencies was calculated by the 'chi-square' test.

Esterase Isoenzyme studies:

The following varieties were used for studying the variation in esterase isoenzymes:

TABLE - 2

Sl. No. (1)	Variety (2)	Plant ht. (CM'S) (3)	Nature of aroma (4)	Source (5)
1.	Type-3	98.0	MH	I.A.R.I.New Delhi
2.	Kalanamak	115.0	MH	-do-
3.	Basmati-370	93.0	MH	-do-
4.	Kanakjeer	88.0	MH	-do-
5.	Improved Sabarmati	80.5	ML	-do-
6.	Badshabhog	103.0	ML	-do-
7.	Gopalbhog	112.5	ML	-do-
8.	Desi Basmati	103.0	ML	-do-
9.	HR-97	86.0	MS	-do-
10.	Chandanchur	130.5	LS	-do-
11.	Assam 634	91.0	LS	-do-
12.	Bindli	104.5	MS	-do-
13.	Habganj Bora II	85.0	MH	-do-
14.	Randhunipagalu	105.5	HS	C.R.R.I., Cuttack
15.	Godavari Isuka	110.0	MH	-do-

(1)	(2)	(3)	(4)	(5)
16.	NP-112	124.5	MH	C.R.R.I., Cuttack
17.	Krishnakatukalu	129.5	MH	-do-
18.	Likitimachi	121.8	MH	-do-
19.	Govindabhog	148.5	ML	-do-
20.	Seetabhog	103.5	LS	-do-
21.	Ambemohar 157	121.0	LS	-do-
22.	Pae Ikulako	143.5	ML	I.R.R.I., Philippines
23.	Basmati Surkh 89-15	122.5	MS	-do-
24.	Basmati Surkh 161	123.0	ML	-do-
25.	Basmati 122	103.5	LS	-do-
26.	Basmati 377	111.5	LS	-do-
27.	NP 119	110.0	MH	C.R.R.I., Cuttack
28.	SO-19	83.0	LS	A.P.A.U., Hyd.
29.	Basmati-local	96.5	MS	Local collection from Warangal Dist. A.P.
30.	Basmati-Warangal	95.5	MS	
31.	Tellahamsa	80.5	NS	Germ plasm collection of Plant Genetics Section, Dept. of Genetics., O.U.
32.	DGWG	95.0	NS	
33.	TKM 6	105.0	NS	
34.	IET-1444	75.5	NS	
35.	Bala	90.0	NS	C.R.R.I., Cuttack
36.	Ratna	95.0	NS	

HS = High scented, MH = Medium high scented
MS = Medium scented, ML = Medium low scented
LS = Low scented, NS = Non-scented

Disc-Electrophoresis was used in the present study (Davis 1964).

Gels were cast in cylinderical glass tubes, which were of 10 cm. length and 0.5 cm in internal diameter. These tubes were fixed in a stand and 40% sucrose solution (w/v) was carefully poured into these tubes with the help of a syringe and capillary tube, to a height of 0.5 cm. This served as the spacer gel. On top of this, large pore solution was placed to a length of 1.0 cm. with the help of a syringe and capillary tube. Distilled water (2-3 drops) was added to avoid the formation of meniscus. Large pore solution contained the following solutions:

a) Tris-hydroxy methyl amino methane 5.98 gms, Tetra methyl ethylene diamine 0.46 ml., 1N HCl 48 ml. made to a final volume of 100 ml, with distilled water (pH 6.7).

b) Acrylamide 10 gms, 'NN' methlene bis acrylamide 2.5 gms in a final volume of 100 ml.

c) 0.004% Riboflavin solution.

d) 40% sucrose solution.

in the proportion of 1:2:1:4 respectively. This solution was exposed to fluorescent light for photopolymerisation, which usually lasted for about 15 minutes.

Water was removed with a syringe and the adhering water droplets were removed by adsorbing onto filter paper strips. Now small pore gel solution was added to completely fill the tubes and water was added again on top of this to avoid meniscus formation. Small pore gel solution contained the following solutions:

a) Tris hydroxy methyl amino methane 36.0 gms, Tetra methyl ethylene diamine 0.23 ml., 1N HCl 48 ml. in a final volume of 100 ml.(pH 8.9).

b) Acrylamide 28.0 gms, N'N' methylene bis-acrylamide 0.735 gms. in a final volume of 100 ml.

c) Distilled water.

d) 0.14% Ammonium persulfate solution.

in the proportion of 1:2:1:4. The gel tubes were kept in subdued light for about 45 minutes to polymerise the small pore gel. After this, the tubes were fixed in the electrophoresis chamber for further use. A buffer solution containing 0.05 M Tris hydroxy methyl aminomethane and 0.038 M Glycine (pH 8.3) was used as the Electrolyte buffer. Bromophenol blue was used as the tracking dye.

Seeds were surface sterilized with 0.1% Sodium hypochlorite solution, and were kept for germination

in glass vials. 5 day old seeldings, were used in the present study.

Tissues were extracted in a spot-plate in cold conditions (4-8°C) in 0.5 ml. of Tris-Glycine buffer (pH 8.9). Whatman No.1. filter paper discs were punched out to fit in exactly into the gel tubes and the enzyme extract was adsorbed on to these discs. Two discs were used for each gel tube. Electrophoresis was done in a cold chamber (6-8°C) at a constant current of 3 mA per tube, which usually lasted for about 1½ to 2 hours. The gels were removed from the tubes, with the help of a syringe needle. These gels were rinsed with distilled water and were stained for esterase activity (Payne and Koszykowski 1978). Staining solution contained 75 mg. of Fast Blue RR salt, 2.25 ml. of substrate solution (1% of 1-naphthyl acetate in 50% acetone) in 150 ml. of 0.6 M phosphate buffer at pH 6.1. The gels were completely covered with the stain and were incubated in the dark for 1 hour at 37°C. After incubation, the stain was removed and gels were rinsed in distilled water and were fixed in 7% acetic acid solution. After fixing they were stored in 2% acetic acid solution. The Rf values were taken for the different

bands and they were calculated as follows:

$$\text{Rf value of the band} = \frac{\text{Distance travelled by the particular band}}{\text{Distance travelled by the tracking dye.}}$$

The gels were scanned for the anzyme activity with a Chromoscan mark II (Joyce Lobel) recording integrating densitometer. The activity of each band was expressed as a percentage of the total activity of all the bands, calculated from the area under the curve recorded for each of the bands.

Lipoxygenase enzyme studies:

This enzyme was studied quantitatively in the following varieties.

1. Randhunipagalu	2. Godavari isuka
3. Type-3	4. Pokura
5. Improved Sabarmati	6. NP 119
7. HR 97	8. Ambemohar
9. Basmati-Warangal	10. Basmati 427
11. IR-8	12. Tella Hamsa

Of these, varieties 1 to 10 are scented and 11 & 12 are non-scented.

Quantitative assay:

(a) Extraction: Dehusked rice was freshly powdered and this was used for the extraction. 25 gm. of this powder

was stirred with 100 ml. of 0.05 M Phosphate buffer (pH 7.0) for 30 minutes at 3-5°C. After extraction, the slurry was filtered through, 2 layers of cheese cloth and finally centrifuged at 4°C at 10,000 g for 15 minutes. The resulting supernatant was used as the enzyme source.

(b) Assay: The method of Surrey (1964) was followed for the quantitative assay.

Linoleic acid substrate was prepared in the following manner: 0.5 ml. of Tween 20 (Sigma) was dissolved in 10 ml. of 0.2 M Borate buffer (pH 9.0) and 0.5 ml. of linoleic acid (> 99% Sigma) was added drop by drop. This suspension was ultrasonically dispersed in a 'Mullard' sonifier, to disperse the acid into a fine emulsion. To this solution, 1.3 ml. of 1 N NaOH solution was added and the mixture was once again agitated to get a clear transparent solution. To this solution 90 ml. of Borate buffer was added and the final volume was made upto 200 ml. with redistilled water. This solution was approximately 7.5×10^{-3}M in linoleic acid and 0.25% in linoleate and Tween 20. Finally the solution was adjusted to the desired pH with concentrated HCl.

The enzyme extract of 1 ml. was taken in a test tube and 8 ml. of substrate was added to it. Oxygen was passed continuously into the test tube during the tenure of the experiment. From this reaction mixture, 1 ml. aliquots were transferred to test tubes containing 2 ml. of absolute alcohol at different time interval viz., 5,10,15 and 20 minutes. 7 ml. of 60% alcohol was added to these tubes, to make a total volume of 10 ml. Control solution was prepared, by using the same quantities of substrate and enzyme, but first the enzyme was added to 2 ml. of absolute alcohol, mixed and was allowed to stand for a few minutes. To this 60% alcohol was added and then substrate was added. Duplicate samples were assayed in all the cases.

Clear alcoholic solutions were taken for reading the optical densities. 2 ml. of the solution was taken in a cuvette (10 mm light path) and the increase in optical density was read at 234 nm, against the control solution in a 'EC' Spectrophotometer.

Effect of pH and time on the activity of the enzyme:

The enzyme was tested at different pH levels, viz., 5,6,7,8,9,10 and at different time intervals, viz., 5,10, 15 & 20 minutes, to study the optimum

pH and time. For studying the effect of time on the enzyme, aliquots at different time intervals were taken at the optimum pH value and the optical densities recorded.

The activity was expressed in terms of 'Units'. One unit causes an increase in A_{234} of 0.001 per minute at room temperature and the specific activity was expressed in terms of units/mg protein.

Protein in the enzyme extract was measured by the Biuret method, following Gornell et al (1949). 1 ml. of the enzyme extract was taken in a test tube and to it 3 ml. of Biuret reagent was added. This was allowed to stand for at least 30 minutes, and after this the increase in optical density was measured at 540 nm against control solution, in a 'Spectronic 20' colorimeter. Control solution was prepared by adding 1 ml. of water and 3 ml. of Biuret reagent.

Bovine serum albumin (Sigma) was used as the standard protein.

Flavor component studies:

The following varieties were used for the study of cooked volatile flavor compounds.

1.	Randhunipagalu	Highly Scented
2.	Basmati - 370	Medium Scented
3.	Likitimachi	-do-
4.	Desi Basmati	-do-
5.	Type - 3	-do-
6.	Godavari Isuka	-do-
7.	Basmati Sufaid 106	Low Scented
8.	Sabarmati	-do-
9.	Jaya	Non Scented
10.	Tellahamsa	-do-

Method of extraction of volatiles:

For the extraction of volatile flavor compounds from cooked rice, the method of Likens and Nickerson (1970) was adopted. This method is best suited for the extraction of volatiles from cooked foods as it allows a simultaneous extraction and concentration of the volatile flavor components. The apparatus contains a three necked flask in which the substance is cooked by steam coming from an inlet. The vapors are led into a jacket cooled with chilled water. Solvent and flavor compounds interact here and the flavor components are bound to the solvent.

250 grams of dehusked rice is taken in a 2500 ml. three necked flask. To it 400 ml. of glass-distilled water is added and the flask in stoppered. The flask is heated to about 60-70°C and simultaneously steam supply is also started. After the entry of steam, heating of the flask is discontinued and the rice is allowed to cook by the steam. As the rice is being cooked, the liberating volatiles are swept by the steam into the extractor. Here the volatiles are extracted by the solvent and are condensed by the chilled condenser. The volatiles along with the solvent collect in the solvent flask, and are collected from there. Either Isopentane (20-25°C) or Pentane (30-35°C) were used as the solvents. Each sample of rice was cooked for about 40-50 minutes, to facilitate the complete extraction of volatiles.

Quantification of volatiles: For quantifying the volatiles, the extracted volatiles along with the solvent, from each batch of cooked rice were transferred into a pre-weighed, clean and stoppered weighing flask. The flask was washed thrice with the solvent and the washings were transferred to the weighing flask, to prevent any loss in the flavor components. Now, a

slow stream of dry Nitrogen was passed into the weighing flask, to evaporate the solvent slowly. This was continued till the solvent completely evaporated. Then the weight of the flask was taken and the difference in weights gave the weight of volatiles. To make sure that the solvent evaporated completely, nitrogen was passed into the flask for 2-3 times and final weight of the flask was taken when the values were constant. For each variety, a minimum of 6 batches of rice were extracted for quantification of volatiles. For the rest of the analysis, extracts from 4-5 batches were pooled. The volatiles were suspended in peroxide free Diethyl ether and were stored in a freezer in stoppered test tubes for further use.

GLC of volatiles: The aroma concentrates of volatiles from the varieties, Randhunipagalu, Godavari Isuka, Type-3, Basmati-370 and Jaya were subjected to Gas liquid chromatography in the following manner.

The concentrates of "Randhunipagalu" were subjected to preparative GLC, equipped with "Odour evaluating device" to determine the flavor of separated compounds. 0.6 micro-liters of the concentrate was injected into the GLC and the separating fractions

were smelt by 5 evaluators and the smell of the compound was recorded. The condition of the GLC are as follows:

Model	Shimadzu GC4A
Detector	Thermal conductivity detector
Column	Dual column (Stainless Steel 0.3 mm O.D. x 3 M), packed with 20% Diethylene glycol succinate on chromosorb W (80-100 mesh).
Temperature	Column - 160° Detector - 200° Injection port 200°
Carrier Gas	Helium at the rate of 40 ml/min.
Current in T.C.D.	200 milliampers at an attenuation 1.
Chartspeed	5 mm./min.

The concentrates of 'Randhunipagalu','Godavari Isuka','Type-3','Basmati-370'and'Jaya'were subjected to analytical GLC. 0.5 to 0.7 microliters of the concentrate were injected into the GLC. The condition of the GLC are as follows:

Model	BARC
Detector	Flame Ionisation Detector
Column	Glass column (0.25 mm O.D. x 6 ft.) packed with 10% carbowax 20 M on chromosorb W.
Temperature	Column and Detector 150° Injection port 170°
Carrier Gas	Nitrogen at the rate 30 ml/min.
Chart Speed	0.2 inch./min.

Fatty acid composition studies:

For fatty acid composition studies, the following scented varieties (1-20) and non-scented (21 and 22) varieties were used.

1. Randhunipagalu	2. Godavari Isuka
3. Type - 3	4. Basmati - 370
5. NP 119	6. Habganjbora II
7. HR 97	8. Badshabhog
9. Basmati Sufaid 106	10. Basmati 140
11. Basmati-Warangal	12. Pokura
13. Chandanchur	14. Basmati 427
15. Ambemohar	16. Kalanamak
17. Likitimachi	18. Improved Sabarmati
19. Desi Basmati	20. Gopalbhog
21. IR - 8	22. Tellahamsa

Extraction of lipids:- Lipids were extracted according to the method of Lee et al (1965). 50 gms. of the dehusked rice was freshly powdered, and was immediately extracted with the solvent, to prevent any loss in the yield of lipids due to the action of lipolytic enzymes. Chloroform:Methanol (2:1v/v) was used as the solvent and duplicate samples were analysed for each variety. Powdered rice sample was taken in an Omni-Mixer and 200 ml. of the solvent was added to the sample. The contents were thoroughly agitated by operating the mixer at medium speed

for 5 minutes. The contents were transferred to a Buchner funnel and the solvent was filtered under vaccum. The rice sample was resuspended in fresh solvent and extracted again in a similar fashion. In this way, each sample was extracted for a minimum of 3 times, to ensure complete extraction of the lipids. The extracts were pooled and transferred to a separating funnel. Separation of the miscible chloroform:methanol extract into two immiscible layers, with subsequent partitioning of the lipids into the chloroform layer, was achieved by the addition of 0.2 M magnesium chloride solution in the proportion of 1 volume of magnesium chloride solution per 5 volumes of the lipid extract. The separating funnels were transferred to a cold room and partitioning was allowed to proceed for 24 hours. After complete partitioning, the lipid free upper layer was discarded and the lower lipid extract was taken in a rotary thin-film evaporator to remove the solvent. The lipid residue was transferred to pre-weighed, stoppered weighing flask, and the last traces of the solvent were removed by blowing a slow stream of nitrogen. The flask was weighed along with the lipid to determine the yield of lipid. Average of duplicate samples

were taken as the final weight of the lipid. All the lipid samples were made into 10% solutions with chloroform for further use.

TLC of Lipids:- Thin layer chromatographic separation of the lipids was done in the following manner. Glass plates (20 cm x 20 cm) were coated with silica gel (Kieselgel, E. Merck, Type 60) slurry (1:2 w/v in water). The thickness of the gel was 0.5 mm. The plates were activated for 1 hour at 100°C in an oven. After activation, the plates were stored in a dessicator for further use.

0.1 ml. of the lipid extract (10% solution in chloroform) from each variety was spotted on the TLC plate and were placed in the chromatocabs. The chamber was saturated with the solvent system which was, Petroleum ether:Diethyl ether:Acetic acid in the ratio 90:20:1 (v/v). It took about 1 hour for each run.

Two plates were run for each sample. One plate was viewed in a Iodine chamber and the other was sprayed with 50% sulphuric acid and was charred in an oven at 150°C. The position and Rf values were noted after the development of different spots.

Preparation of methyl esters of fatty acids:- About 500 mg's of lipid sample from each variety was transferred to 50 ml. Erlenmeyer flask and 25 ml. of 2N Alcoholic Potassium hydroxide solution was added to each flask. The lipids were saponified by refluing for 2 hours. After saponification, samples were diluted with distilled water to a final volume of 50 ml. and transferred to separating funnels. This solution was washed with Petroleum ether (40-60' boiling range) thrice, to remove non-saponifiables. Now, the samples were treated with 15 ml. of 6 N Hydrochloric acid, to liberate the fatty acids. This mixture was washed with peroxide-free Diethyl ether twice. About 3-4 water washings were given to the ether layer, to remove excess acid. Anhydrous Sodium sulphite was added to the samples to remove the moisture.

The above fatty acid samples were transferred to 100 ml. round-bottomed flasks for methylation. To each sample, 15 ml. of anhydrous Methanol and 2 drops of concentrated sulphuric acid were added and they were refluxed for 2 hours. These samples were extracted with Diethyl ether three times. The ether layer was washed repeatedly with water to remove acid. Three

Three washings with sodium bicarbonate solution (10% w/v) were also given to remove acid, and finally the mixture was washed with water to remove excess alkali. The ether layer was transferred to a flask and anhydrous sodium sulphite was added to remove moisture.

These samples were evaporated in a rotary, thin film evaporator and the last traces of the solvent were removed by blowing nitrogen. The samples were transferred to stoppered tubes and were stored in a refrigerator for further use.

Gas chromatographic analysis:- For determination of the fatty acid composition, a gas chromatograph, with the following conditions was utilised:

Model	F & M
Detector	Flame ionisation detector
Column	Stainless Steel column (183 cm x 0.635 cm. OD) packed with 10% ethylene glycol succinate.
Temperature	Column 210°C Injection port and detector 300°C.
Carrier Gas	Nitrogen at a flowrate of 2400 ml./hr.
Chart Speed	60 cm./hr.

0.5 micro liters of the fatty acid mixture was injected from each sample. The fatty acids were identified by comparision of their retention times with those of reference standards. Gas chromatographic areas were measured by multiplying peak height by peak width at half height. Means of duplicate samples were taken as the final percentage.

Amino acid composition studies:-

The following varieties were used for studying the amino acid composition:

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3
4. Basmati - 370
5. Seetabhog
6. Likitimachi
7. Kanakjeer
8. Ambemohar
9. Bindli
10. SO - 19
11. Basmati 93 B
12. Basmati Sufaid 100
13. Tellahamsa.

Hydrolysis:- Uniform seeds of different varieties were dehusked and ground to a fine powder. 100 mg $\pm$ 2 mg. of this powder was transferred to a hydrolysis tube and 3 ml. of 6N HCl (constant boiling mixture) was added to it. The ampoule was attached to a high vaccum pump for evacuation of the ampoule. The neck of the ampoule was sealed in vaccum by means of a

blow torch. The tubes were kept in a hot-air oven at 110°C ± 1°C for 24 hours, to effect the hydrolysis. At the end of hydrolysis, the contents of the ampoules were quantitatively transferred to 25 ml. beakers and these beakers were kept in a vaccum dessicator over NaOH pellets and P_2O_5 for the removal of HCl. The samples were brought to dryness after 2 to 3 washings with distilled water, to ensure complete removal of HCl. After this, the contents were made upto 15 ml. with citrate buffer (pH 2.2). These were centrifuged at 10,000 g to remove the debris and were transferred quantitatively into clean test tubes for further use. All the samples were either colorless or faint yellow in color.

Amino acid analysis:- This was accomplished on a JEOL JC-6 amino acid analyser. 1 ml. of the sample was loaded onto each of the columns. The resin used was LC-R2. The chromatograms were integrated manually by the height x width method, and the amino acids were calculated in term of micro moles and then converted into percentage basis of protein. Tryptophan and cysteine were not estimated.

Protein content of the varieties:- Protein in the samples was estimated by the micro-kjeldhal method. 50 mg. ± 1 mg. of the finely ground rice powder was taken in a micro-kjeldhal flask and was digested over heat with 3 ml. of conc. sulfuric acid and catalyst (98 parts $K_2 SO_4$ + 2 parts $Cu SO_4$ + 1 part Selenium dioxide). After digestion, the sample was transferred to micro-kjeldhal distillation unit and the ammonia was liberated and adsorbed into a conical flask containing 10 ml. of boric acid, bromo-cresol green indicator. Heating was continued till the indicator turned blue and about 25 ml. of the distillate was collected. This was titrated against 0.01 N Sulfuric acid till the end point (Purple color) reached. The percentage of the protein was calculated as follows using the conversion factor 5.95.

$$\% \text{ Protein } \frac{(x-y) \times b \times 0.14 \times 5.95 \times 100}{a \times 0.01 \times 1000}$$

where x = Titre value of the sample.
y = Titre value of the blank.
b = Normality of the sulphuric acid used.
a = Weight of sample (gms.).

Duplicate samples were run for each variety and the mean values were taken as the final percentages.

OBSERVATIONS

I. Genetic studies:

The inheritance pattern of 'aroma' in rice, was studied among the segregating material of six crosses. The results are presented in the Table 3. In the crosses involving scented and non-scented rice varieties, viz., Randhunipagalu (Scented) x TN (1) (Non-scented), Randhunipagalu (Scented) x IR 8 (Non-scented), Pokura (Scented) x Deepaiguda (Non-scented), Bala (Non-scented) x Pokura (Scented), Type - 3 (Scented) x Bala (Non-scented), all the F_1 plants were non-scented. In the cross with scented varieties i.e., Basmati - 370 (Scented) x Type - 3 (Scented), however the F_1 plants were scented.

In the F_2 generation, about 170 to 220 plants from each cross were analysed for the presence or

absence of 'aroma', by treating the leaf material to 1.7% Potassium hydroxide solution and observing the scentedness. The nature of aroma was confirmed in the grains also. In the crosses involving scented x non-scented rice varieties, the F_2 plants clearly segregated into scented and non-scented ones. The data was analysed which exhibited a monogenic ratio of 3 : 1 (non-scented : scented) The data was subjected to chi-square test. All the F_2 plants in the cross Basmati - 370 x Type 3 (scented) gave scented only.

TABLE - 3

AROMA PATTERN IN F_1 & F_2 POPULATIONS OF DIFFERENT CROSSES

Sl. No.	C R O S S	F_1	F_2	No. of Plants				X^2	P Value
				Non-Scented		Scented			
				Obse-rved	Expe-cted	Obse-rved	Expe-cted		
1.	Randhuni Pagalu x TN(1)	Non-scented	180	131	135	49	45	0.474	0.5-0.3
2.	Randhuni Pagalu x IR 8	-do-	188	136	141	52	47	0.709	0.5-0.3
3.	Pokura x Deepaiguda	-do-	204	150	153	54	51	0.235	0.7-0.5
4.	Bala x Pokura	-do-	220	163	165	57	55	0.096	0.8-0.7
5.	Type-3 x Bala	-do-	196	150	147	46	49	0.245	0.7-0.5
	Total		988	730	741	258	247	1.759	

X^2 for 1 d.f. at 0.1% level of significance is 2.706.

II. Esterase isoenzyme studies:

Esterase isoenzymes were studied in five day old seedlings of thirty scented and six non-scented rice varieties (Fig. 1 a,b,c). The presence of specific bands is shown in Table 4. Fig. 2 (a.:.f) represents the zymograms along with densitometer scans of the gels. The activity per isozyme band exhibited as percent total activity is shown in Table 5. A total of 14 bands were present in the zymograms. The Rf values ranged from 0.06 to 0.75, indicating that bands were present in all the regions of mobility, i.e., from slow to fast migrating bands. All the varieties showed band 6(Rf 0.24), band 9 (Rf 0.51) and band 10 (Rf 0.53). Of these, band 6 was of medium stainability and bands 9 and 10 were darkly stained. The above visual observations correlated with the densitometer scans of the gels. The percent total activity of band 6 ranged between 1.13 to 10.10, whereas bands 9 and 10, showed the activity around 14.32 to 24.30 and 18.00 to 32.00 respectively.

The varieties were classified into five groups, based on their nature of aroma (Table 2). They are:

(a) high and medium-high scented, (b) medium-scented, (c) medium-low scented, (d) low-scented and (e) non-scented.

The varieties of group 'a', Randhunipagalu, Godavari Isuka, Type-3, NP-112, Basmati-370, Kalanamak, Habganjbora II, Kanakjeer, Likitimachi, NP-119 and Krishnakatukalu showed similar types of zymograms. All of them exhibited, band 2 (Rf 0.09) with the exception of Randhunipagalu, Godavari Isuka, NP-112 and Basmati-370, band 3 (Rf 0.12) with the exception of Habganjbora II, whereas band 5 (Rf 0.19), band 6 (Rf 0.24), band 9 (Rf 0.51), band 10 (Rf 0.53) and band 12 (Rf 0.65) were present in all the varieties. The densitographic analysis of the gels revealed that the bands 9, 10 and 12 were the major bands in this group, followed by bands 3,5 and 6. All the varieties were characterised by the presence of a darkly stained band 12 (Rf 0.65) whose activity ranged from 19.80 to 34.45. The activity of band 3 varied from 1.46 to 16.43.

The varieties of group 'b', Bindli, Basmati-Warangal, Basmati-local, HR-97 and Basmati surkh

TABLE - 4

PRESENCE OF ESTERASE ISOENZYME BANDS IN DIFFERENT RICE VARIETIES

Sl. No.	Variety	0.06	0.09	0.12	0.14	0.19	0.24	0.27	0.46	0.51	0.53	0.63	0.65	0.68	0.75	Rf value Band Nos.
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1.	Randhunipagalu			+		+	+			+	+		+			
2.	Godavari Isuka			+		+	+			+	+		+			
3.	Type 3		+		+	+	+			+	+		+			
4.	NP 112			+		+	+			+	+		+			
5.	Basmati 370			+	+	+	+			+	+		+			
6.	Kalanamak		+	+		+	+			*	+		+			
7.	Habzganj Bora II		+		+	+	+	+		+	+		+			
8.	Kanakjeer		+	+		+	+			+	+		+			
9.	Likitimachi		+	+		+	+			+	+		+			
10.	NP 119		+	+		+	+			+	+		+			
11.	Krishnakatukalu		+	+		+	+			+	+		+			
12.	Bindli	+		+			+			+	+				+	
13.	Basmati Warangal	+		+			+	+		+	+				+	
14.	Basmati local	+		+			+			+	+				+	
15.	HR 97	+				+	+			+	+				+	
16.	Basmati Surkh 89-15		+			+	+	+		+	+			+	+	
17.	Desi Basmati		+	+		+	+	+		+	+			+	+	
18.	Basmati Surkh 161		+		+	+	+			+	+			+	+	

TABLE - 4 (Contd.)

Sl. No.	Variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14
19.	Improved Sabarmati		+	+	+	+	+			+	+			+	+
20.	Badshabhog		+	+			+	+		+	+			+	+
21.	Pae Ikulaku		+		+	+	+	+		+	+			+	+
22.	Gopalbhog		+		+	+	+	+		+	+			+	+
23.	Govindabhog		+	+			+	+		+	+			+	+
24.	SO 19		+			+	+			+	+			+	+
25	Basmati 377		+			+	+			+	+				+
26.	Seetabhog	+		+			+			+	+			+	+
27.	Basmati 122	+				+	+			+	+				+
28.	Assam 634	+		+			+			+	+				+
29.	Ambemohar	+		+			+			+	+				+
30.	Chandanchur	+		+			+			+	+				+
31.	Tellahamsa			+			+	+	+	+	+	+		+	+
32.	DGWG				+	+	+		+	+	+	+		+	+
33.	TKM 6	+		+			+	+	+	+	+	+		+	+
34.	IET 1444	+		+			+	+	+	+	+	+		+	+
35.	Bala	+		+			+	+	+	+	+	+		+	+
36.	Ratna	+		+			+	+	+	+	+	+		+	+

TABLE - 5

ESTERASE ACTIVITY (RELATIVE PERCENTAGE) IN DIFFERENT VARIETIES OF RICE

Sl. No.	Variety	Bands 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.	Randhunipagalu			16.43		4.21	4.38			18.80	29.54		26.64		
2.	Godavari Isuka			13.71		3.31	3.82			20.42	28.67		30.07		
3.	Type 3		8.39		2.65	6.01	6.07			28.19	31.54		19.80		
4.	NP 112			8.18		4.01	4.16			22.26	41.24		20.13		
5.	Basmati 370			6.23	4.83	5.16	4.52			21.72	23.29		34.25		
6.	Kalanamak		2.14	1.46		1.72	2.57			23.33	43.74		25.04		
7.	Habganzbora II		7.01		10.72	6.63	4.71			22.27	23.71		20.21		
8.	Kanakjeer		3.15	9.91		8.01	10.01			19.26	27.45		22.21		
9.	Likitimachi		7.21	9.61		3.85	2.58			17.46	26.19		33.11		
10.	NP 119		7.82	1.77		19.63	8.16			18.31	16.87		34.45		
11.	Krishnakatukalu		1.81	5.91		1.97	1.31			24.30	32.51		32.18		
12.	Bindli	5.13		43.59		4.03	2.98			8.97	11.54				24.36
13.	Basmati Warangal	11.07		7.47			4.84	3.73		11.89	12.86				48.13
14.	Basmati local	12.83		14.72			1.89			12.08	17.74				40.75
15.	HR 97	7.46				7.40	9.90			11.39	14.85				49.01
16.	Basmati surkh		1.85			7.85	7.50	4.61		15.71	19.64			6.10	36.75
17.	Desi Basmati		18.01	14.09		10.02	7.41			17.12	18.06			0.52	10.86
18.	Basmati surkh														

TABLE - 5 (Contd.)

Sl. No.	Variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14
19.	Improved Sabarmati		10.53		6.22	2.11	0.88			21.93	32.46				25.88
20.	Badshabhog		14.90	25.08			7.61	2.89		16.51	18.85			1.73	12.43
21.	Pae Ikulaku		10.62		15.21	5.73	3.98	0.57		20.63	25.21			1.29	14.36
22.	Gopalbhog		15.29		20.03	4.52	9.47	1.97		14.79	21.85			1.97	11.36
23.	Govindabhog		9.30	11.66			3.68	5.72		20.32	32.43			2.27	7.05
24.	SO 19		1.18			1.32	0.88			24.26	28.41			6.44	37.50
25.	Basmati 377		0.79			0.95	0.81			19.42	30.18				46.19
26.	Seetabhog	5.12		13.93			7.05			23.28	27.51			3.17	19.93
27.	Basmati 122	9.94				7.03	4.68			15.01	20.47				42.88
28.	Assam 634	7.21		11.06			10.10			14.42	21.15				36.06
29.	Ambemohar	6.11		13.74			3.05			20.61	27.48				29.01
30.	Chandanchur	5.11		9.14			1.43			18.57	20.29				42.00
31.	Tellahamsa			6.98			4.72	2.04	3.71	18.21	27.92	3.95		0.91	23.67
32.	DGWG				5.71	3.67	3.61		2.34	20.07	34.11	3.67		1.10	25.72
33.	TKM 6	4.78		6.25			3.72	1.25	2.50	16.25	32.00	5.10		3.90	24.25
34.	IET 1444	10.17		9.73			4.59	1.47	2.12	17.30	25.56	4.75		2.65	21.15
35.	Bala	9.88		6.89			2.12	1.03	2.01	18.21	26.34	4.63		2.03	25.53
36.	Ratna	10.89		8.89			2.22	1.39	3.11	16.44	27.33	4.39		6.28	21.56

showed similar types of electrophoretic pattern. All of them exhibited band 1 (Rf 0.06) except Basmati surkh, band 3 (Rf 0.12) except HR-97 and Basmati surkh, band 6 (Rf 0.24), Basmati-Warangal and Basmati surkh had band 7, band 9 (Rf 0.51), band 10 (Rf 0.53), Basmati surkh had band 13 (Rf 0.68) and all the varieties had band 14 (Rf 0.75). The densitographic analysis showed that bands 3,9,10 and 14 were the major bands in this group, followed by bands 1,5 and 6. All the varieties exhibited a darkly stained band 14, whose activity varied from 24.36 to 40.75. The activity of bands 9 and 10 was slightly less in this group, 11.54 to 24.64 as compared to 18.84 to 28.56 of other groups. The activity of band 1 varied from 5.13 to 12.83.

The varieties of group 'c', Desi Basmati, Basmati surkh 161, Improved Sabarmati, Badshabhog, Pae Ikulaku, Gopalbhog and Govindabhog exhibited similar types of zymograms with a few exceptions. They contained band 2 (Rf 0.09), with the exception of Govindabhog, band 3 (Rf 0.12) with the exception of Basmati surkh 161, Pae Ikulaku and Gopalbhog, band 4 (Rf 0.14) with the exception of Desi Basmati,

Fig. 1 (a) Esterase Isoenzyme pattern in different rice varieties.

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3
4. NP - 112
5. Kalanamak
6. Basmati Surkh 161
7. Krishnakatukalu
8. Basmati - 370
9. Kanakjeer
10. Likitimachi
11. Habganjbora II
12. NP - 119

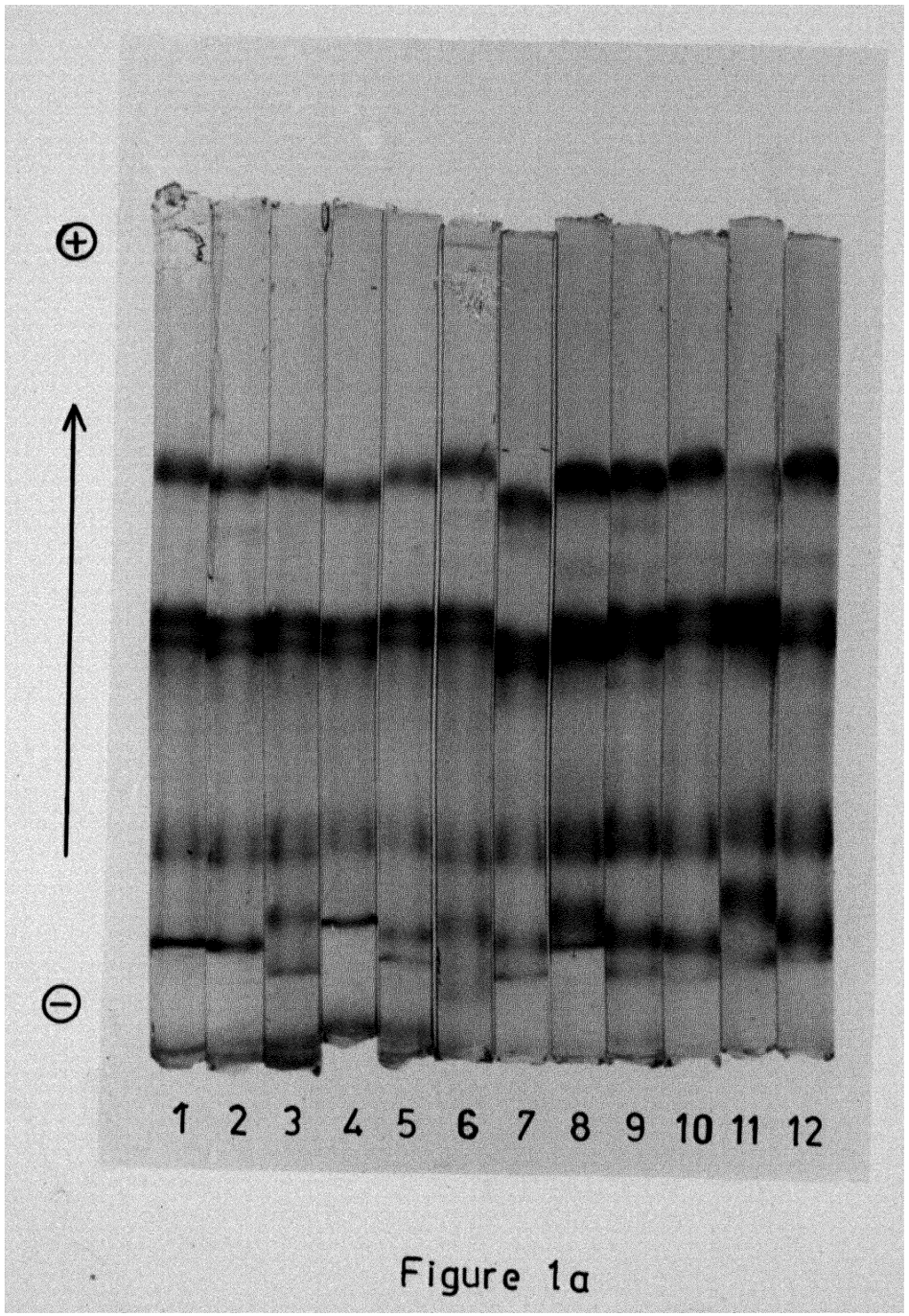


Figure 1a

Fig. 1(b) Esterase Isoenzyme pattern in different rice varieties.

13. Improved Sabarmati
14. Badshabhog
15. Govindabhog
16. Pae Ikulaku
17. Gopalbhog
18. Desi Basmati
19. SO - 19
20. Basmati - 377
21. Basmati Surkh 89-15
22. Seetabhog
23. Basmati - 122
24. HR - 97

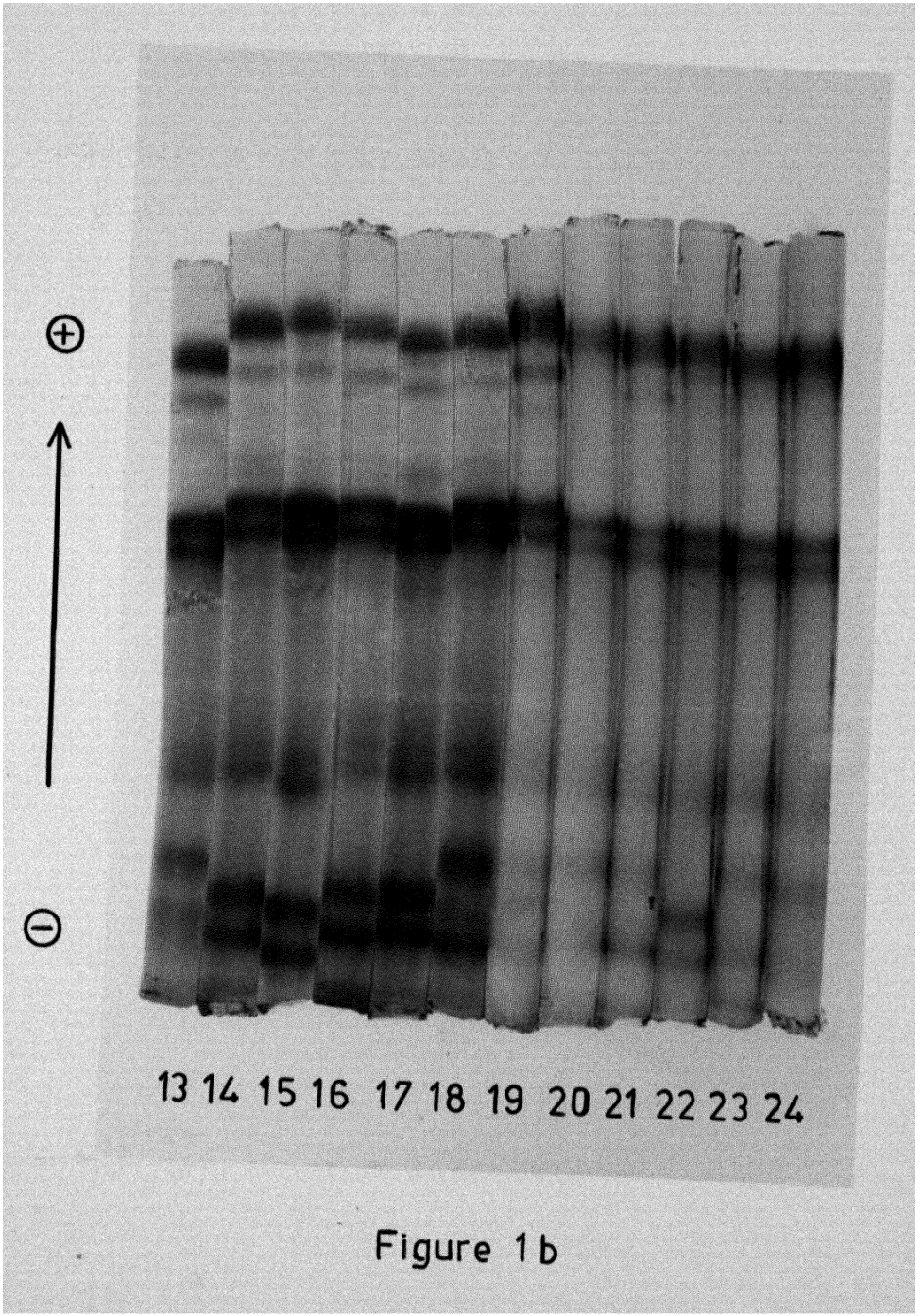


Figure 1b

Fig. 1 (c) Esterase Isoenzyme pattern in different rice varieties.

25. Chandanchur
26. Assam - 634
27. Ambemohar
28. Basmati-local
29. Bindli
30. Basmati - Warangal
31. Tellahamsa
32. DGWG
33. TKM - 6
34. IET - 1444
35. Bala
36. Ratna

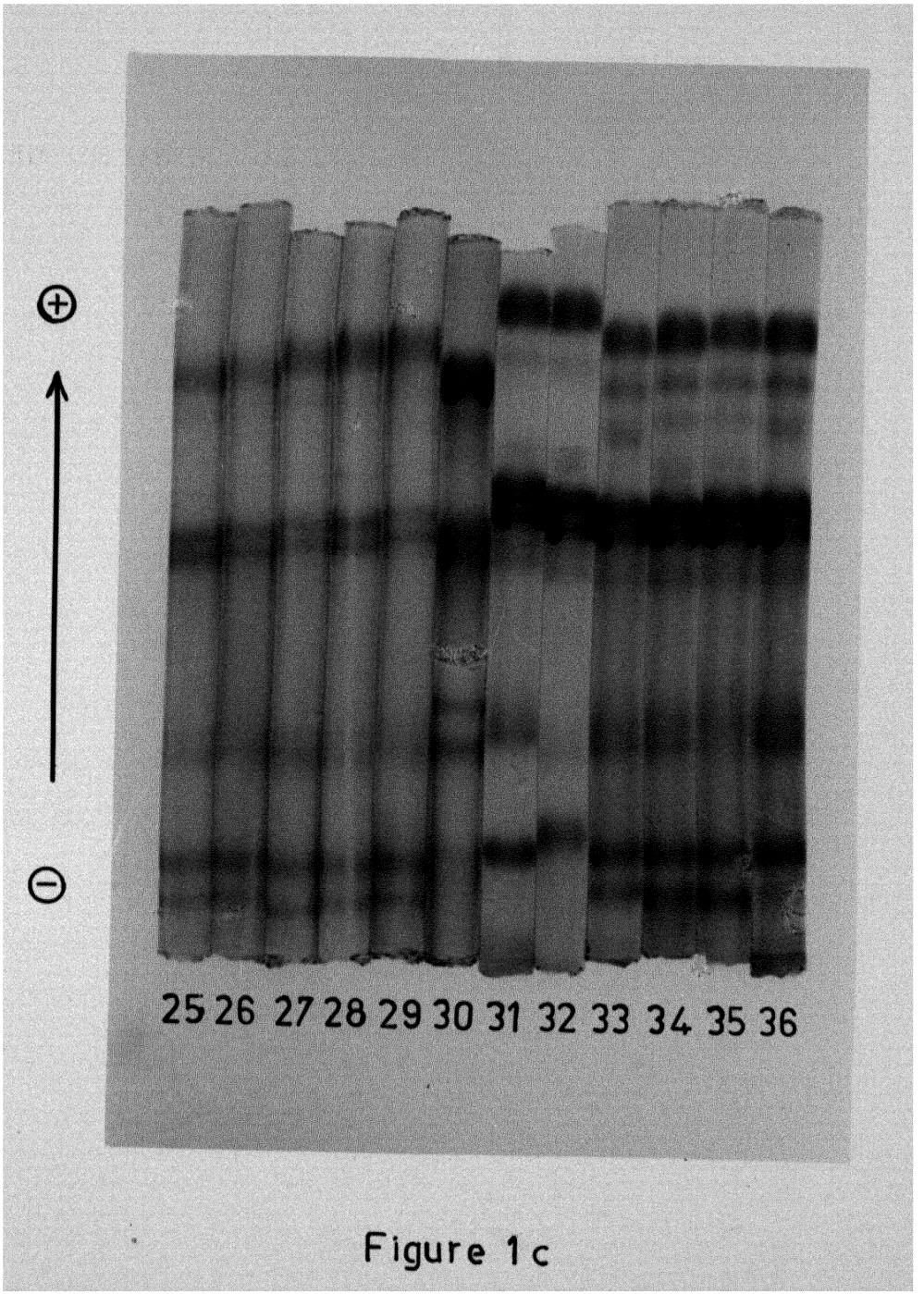


Figure 1c

Badshabhog and Govindabhog, band 5 (Rf 0.19) with the exception of Badshabhog and Govindabhog, band 6 (Rf 0.24), band 9 (Rf 0.51), band 10 (Rf 0.53),band 13 (Rf 0.68) and band 14 (Rf 0.75). The activity of the isozyme bands as shown by the densitograms, revealed that bands 2,9,10 and 14 were the major bands in this group. All these varieties had a lightly stained band 13, which was absent in the groups 'a' and 'b'. The activity of this band ranged from 0.52 to 2.27. The activity of the band 2 was 10.62 to 18.01, band 9 from 14.79 to 21.93, band 10 from 18.06 to 32.43 and band 14 from 7.05 to 12.43.

The varieties of group 'd', SO-19, Basmati 377, Seetabhog, Basmati 122, Assam 634, Ambemohar and Chandanchur exhibited similar types of zymograms. They had band 1 (Rf 0.06) with the exception of SO-19 and Basmati 377 which had band 2 (Rf 0.09), band 3 (Rf 0.12) with the exception of SO-19, Basmati 377 and Basmati 122. SO 19, Basmati 377 and Basmati 122 had band 5 (Rf 0.19), all the varieties had band 6 (Rf 0.24), band 9 (Rf 0.51) and band 10 (Rf 0.53), SO-19 and Seetabhog showed band 13 (Rf 0.68) and all the varieties had band 14 (Rf 0.75). The activity

Fig. 2 (a) Gel scans along with diagramatic representation of banding pattern in different rice varieties.

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3
4. NP - 112
5. Basmati - 370
6. Kalanamak.

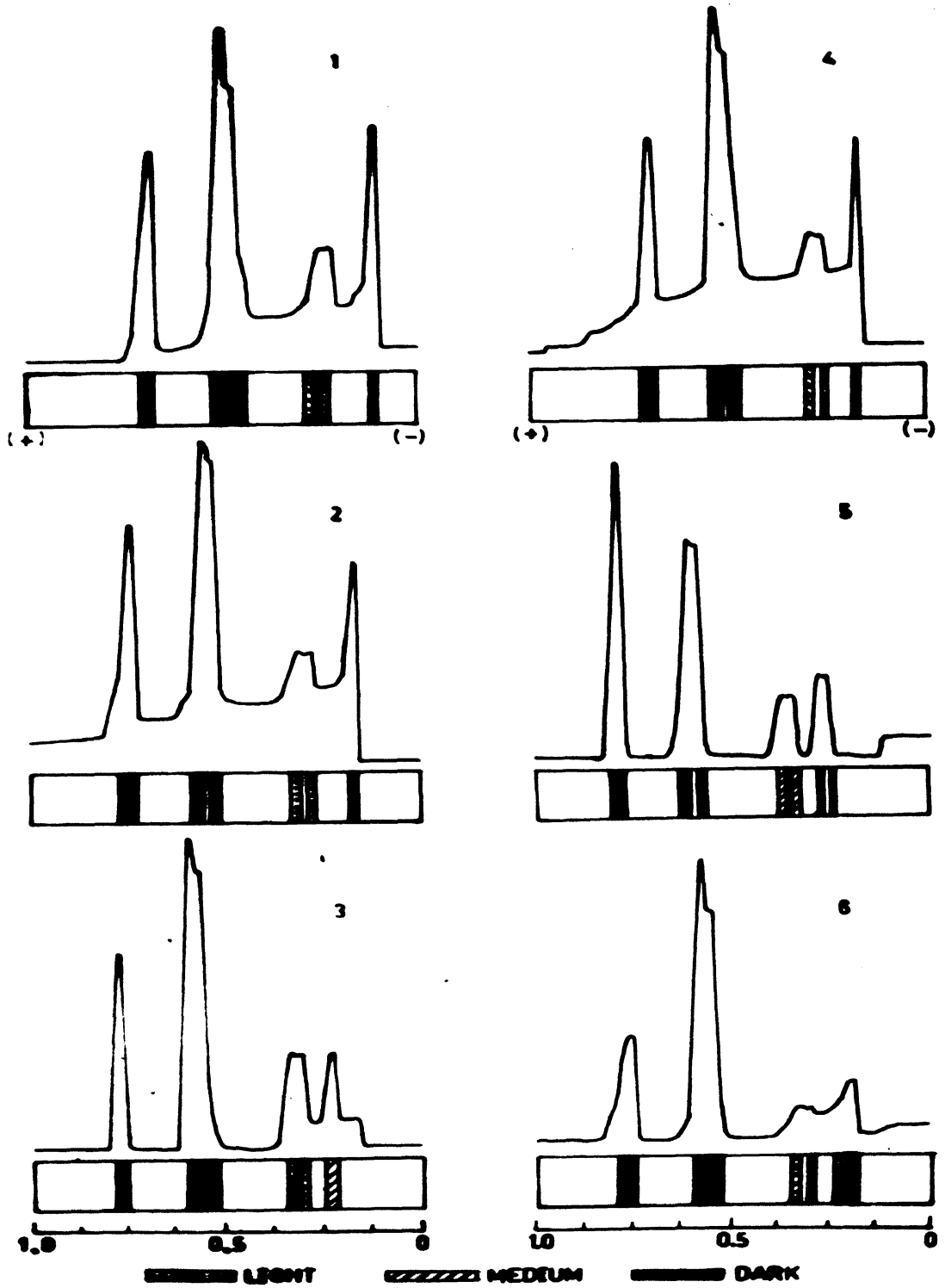


Figure 2a

Fig. 2 (b) Gel scans along with diagrammatic representation of banding pattern in different rice varieties.

7. Habganj Bora II
8. Kanakjeer
9. Likitimachi
10. NP - 119
11. Krishnakatukalu
12. Bindli

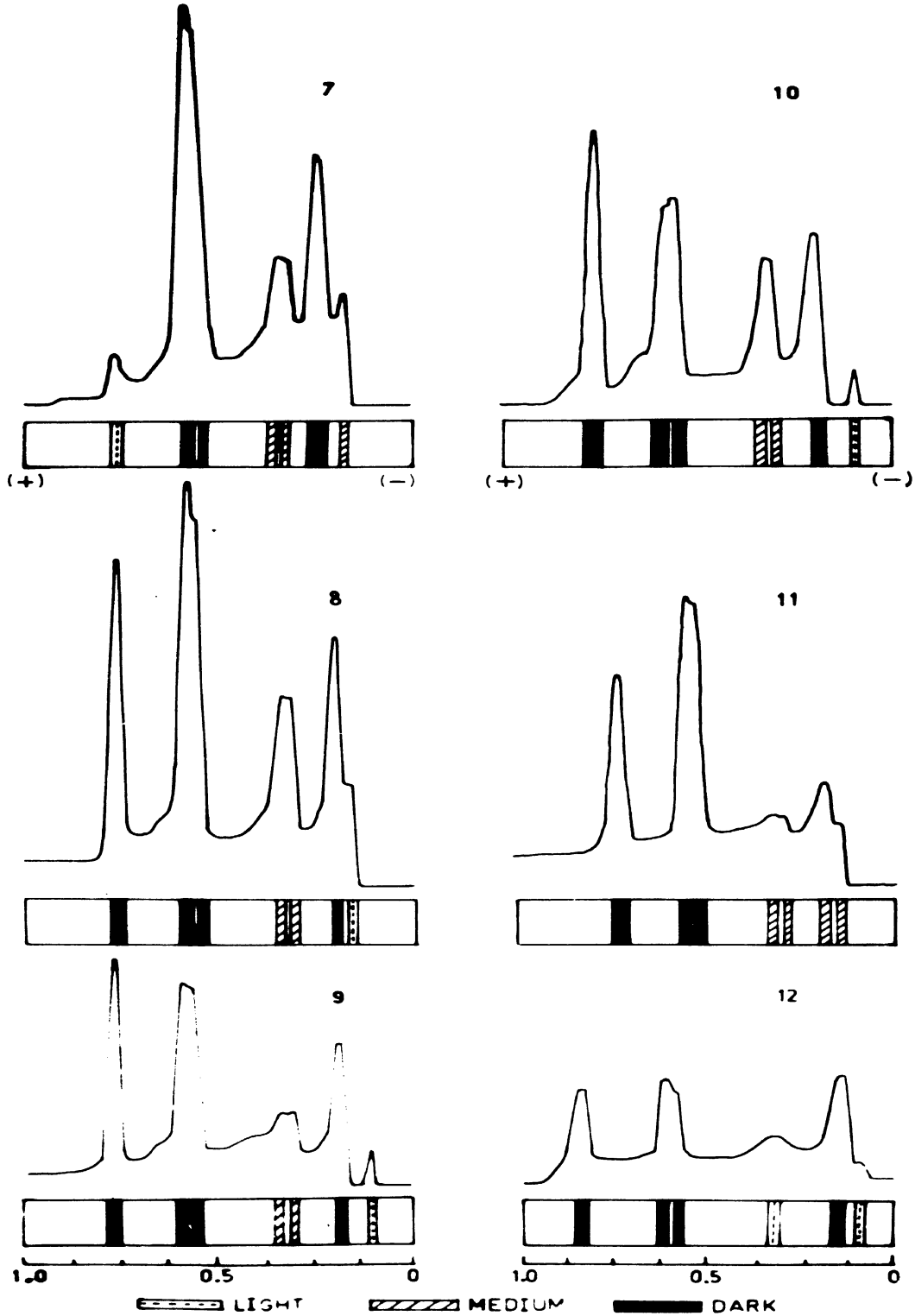


Figure 2 b

Fig. 2 (c) Gel scans along with diagramatic representation of banding pattern in different rice varieties.

13. Basmati - Warangal
14. Basmati - local
15. HR - 97
16. Basmati Surkh 89-15
17. Desi Basmati
18. Basmati Surkh 161

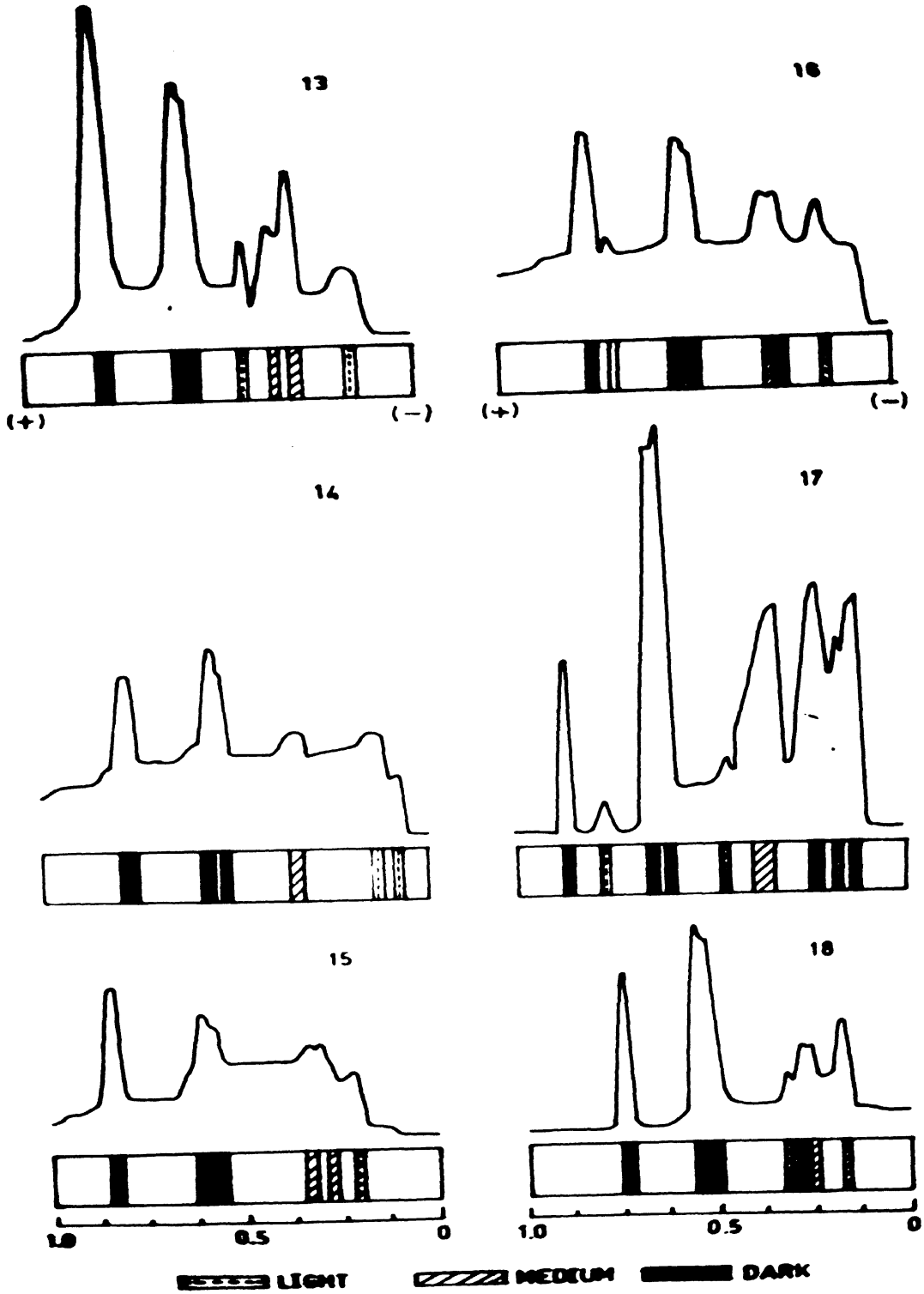


Figure 2 c

Fig. 2 (d) Gel scans along with diagramatic representation of banding pattern in different rice varieties.

19. Improved Sabarmati
20. Badshabhog
21. Pae Ikulaku
22. Gopalbhog
23. Govindabhog
24. SO - 19

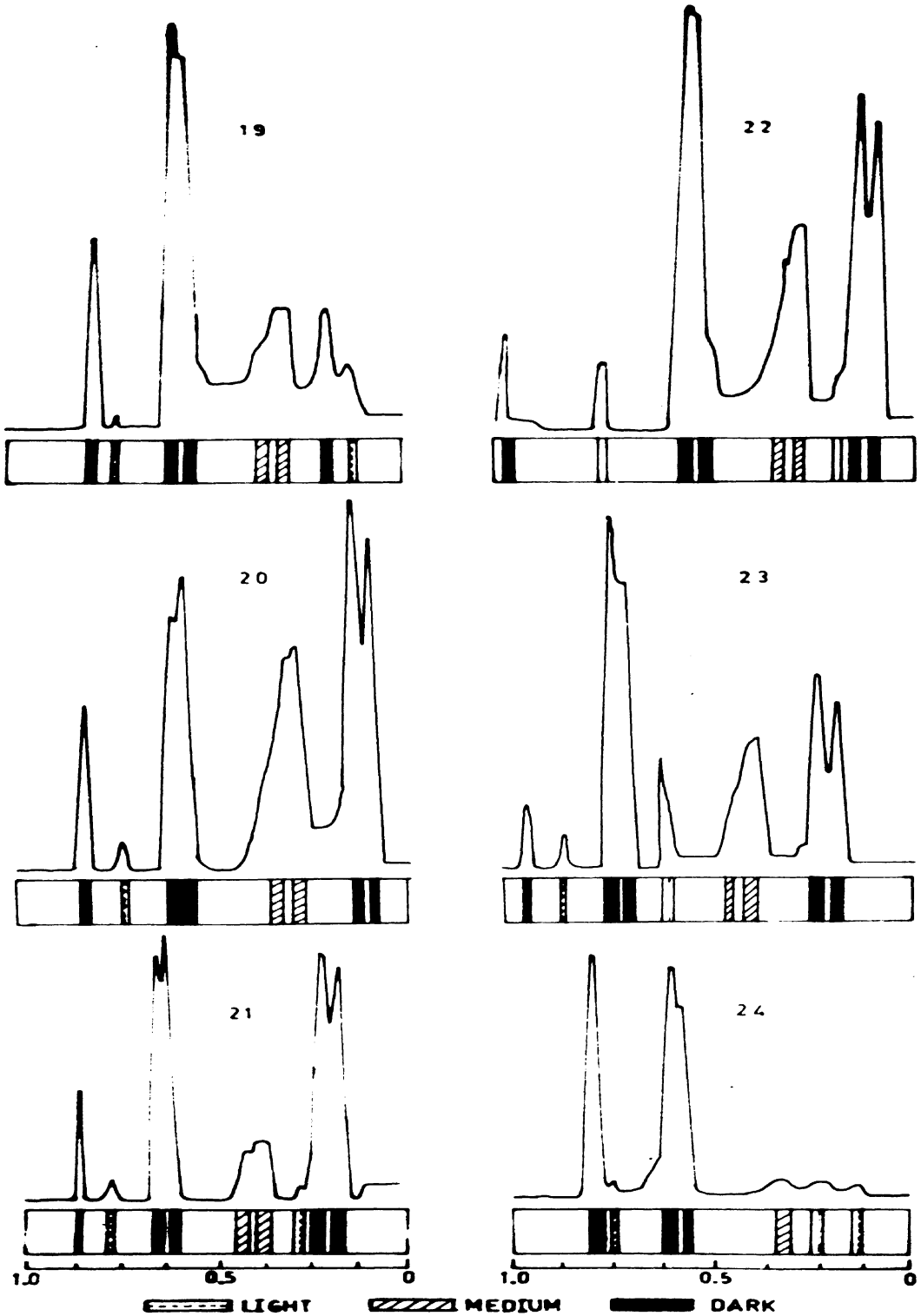


Figure 2d

Fig. 2 (e) Gel scans along with diagrammatic representation of banding pattern in different rice varieties.

25. Basmati - 377
26. Seetabhog
27. Basmati - 122
28. Assam - 634
29. Ambemohar
30. Chandanchur

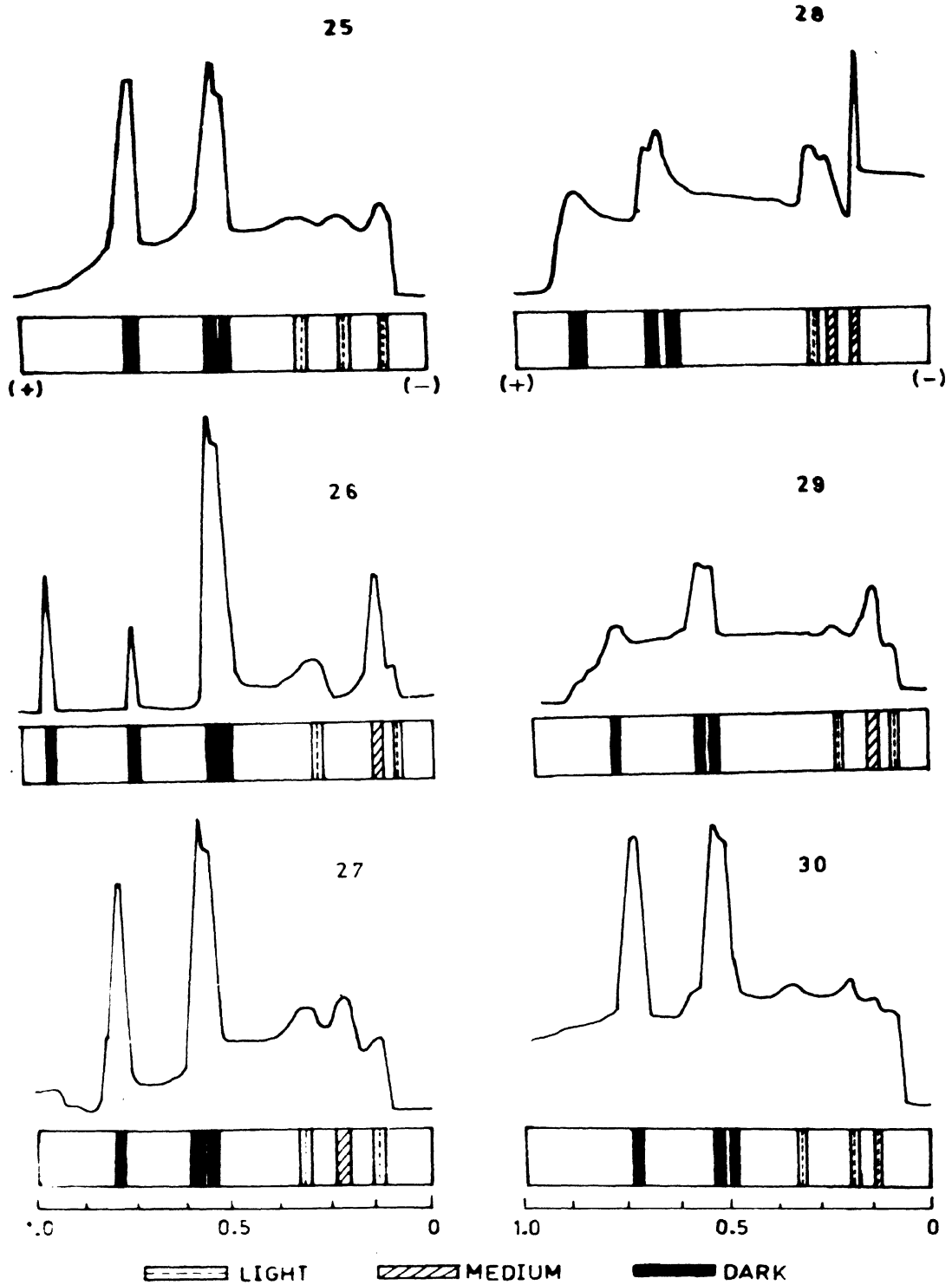


Figure 2e

Fig. 2 (f) Gel scans along with diagramatic representation of banding pattern in different rice varieties.

31. Tellahamsa
32. DGWG
33. TKM - 6
34. IET - 1444
35. Bala
36. Ratna

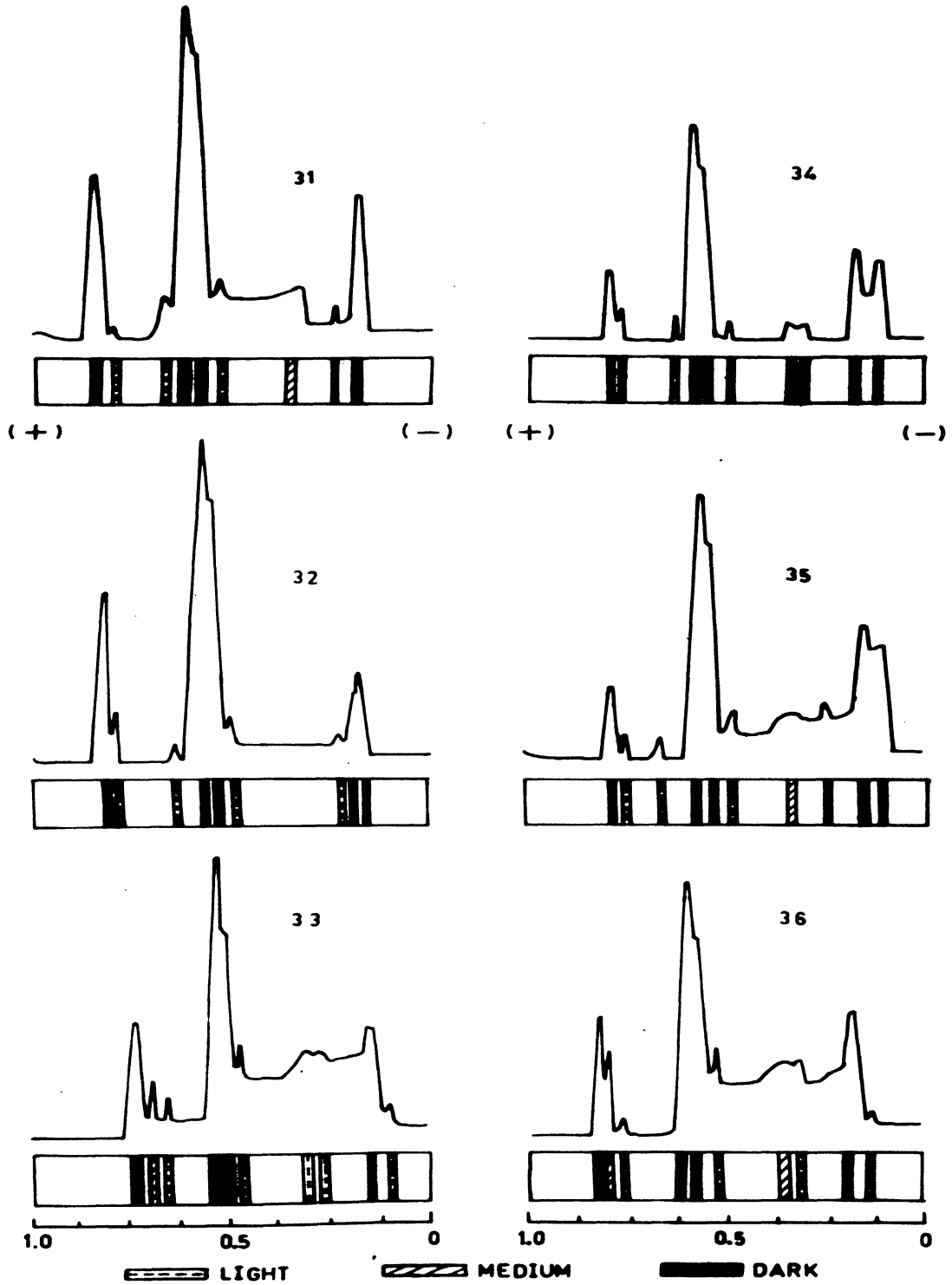


Figure 2 f

of the isozyme bands as shown by the densitograms, revealed that bands 2,9,10 and 14 were the major bands in this group. The activity of the band 2 varied from 10.62 to 18.01, band 9 from 14.79 to 21.93, band 10 from 18.06 to 32.42 and band 14 from 7.05 to 12.43.

All the non-scented rice varieties (group 'e'), Tellahamsa, DGWG, TKM 6, IET 1444, Bala and Ratna showed similar types of electrophoretic pattern, They had band 1 (Rf 0.06) with the exception of Tellahamsa and DGWG, band 3,(Rf 0.12), DGWG had band 4 (Rf 0.14) and band 5 (Rf 0.19), all the varieties had band 6 (Rf 0.24), band 7 (Rf 0.27) with the exception of DGWG, bands 8 (Rf 0.46), band 9 (Rf 0.51), band 10 (Rf 0.53), band 11 (Rf 0.63), band 13 (Rf 0.68) and band 14 (Rf 0.75). All these non-scented varieties had two specific bands, i.e., band 8 and 11 which were absent in all the scented varieties. The activity of isozyme bands as shown by the densitographic scans, revealed that the bands 1,3,9,10 and 14 were the major bands of this group. The activity of band 1 varied from 4.78 to 10.89, band 3 from 6.25 to 9.73, band 8 from 2.01 to 3.11 and band 11 from 3.67 to 5.10.

III. Lipoxygenase enzyme studies:

Lipoxygenase was studied quantitatively in ten scented rice varieties, namely, Randhunipagalu, Godavari Isuka, Type-3, Pokura, Improved Sabarmati, NP-119, HR-97, Ambemohar, Basmati-Warangal, and Basmati 122 and two non-scented rice varieties IR 8 and Tellahamsa.

Effect of pH on the activity of enzyme:- The enzyme extracts from all the varieties were first assayed at different pH levels viz., 5,6,7,8,9 and 10, to know the optimum pH. All the varieties with the exception of IR 8, showed maximum activity around pH 8. The pH curves for these varieties are shown in fig.3a,b,c,d. Although the activities differred, the curves were bell shaped with the maximal activity centered around pH 8 and tapering towards both the sides. Similar results were obtained by Shastry and Rao (1975), who obtained a pH optimum of 8.5 for the lipoxygenase enzyme from rice bran.

The activity of the enzyme from the variety IR 8 was the only exception, where a different type of curve with maximal activity at pH 6 was observed.

Fig. 3 (a) Variation in lipoxygenase activity as a function of pH and time in different rice varieties.

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3

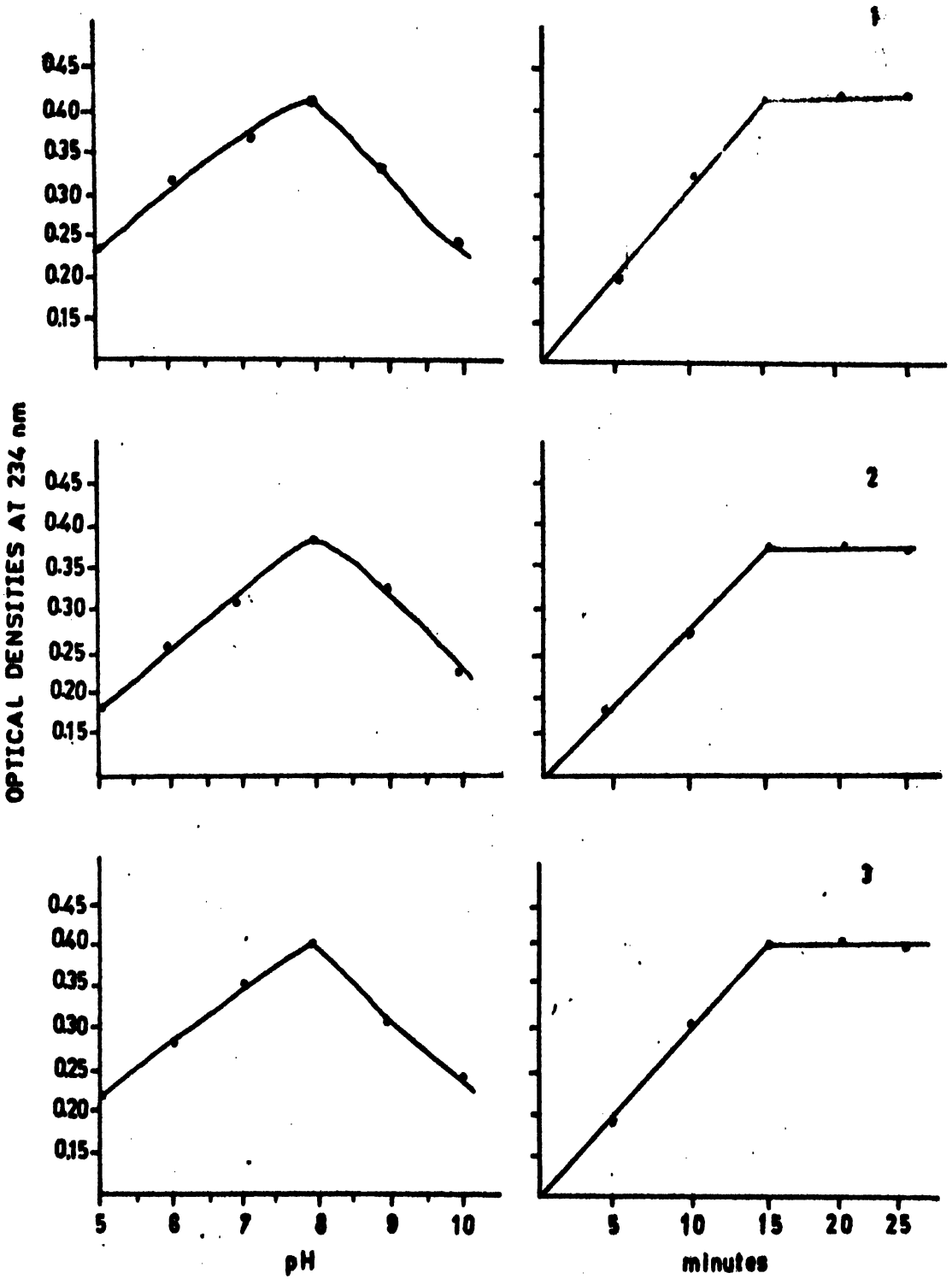

OPTICAL DENSITIES AT 234 nm
0.45
0.40
0.35
0.30
0.25
0.20
0.15
1
2
3
5
6
7
8
9
10
pH
5
10
15
20
25
minutes

Figure 3 a

Fig. 3 (b) Variation in lipoxygenase activity as a function of pH and time in different rice varieties.

4. Pokura

5. Improved Sabarmati

6. NP - 119

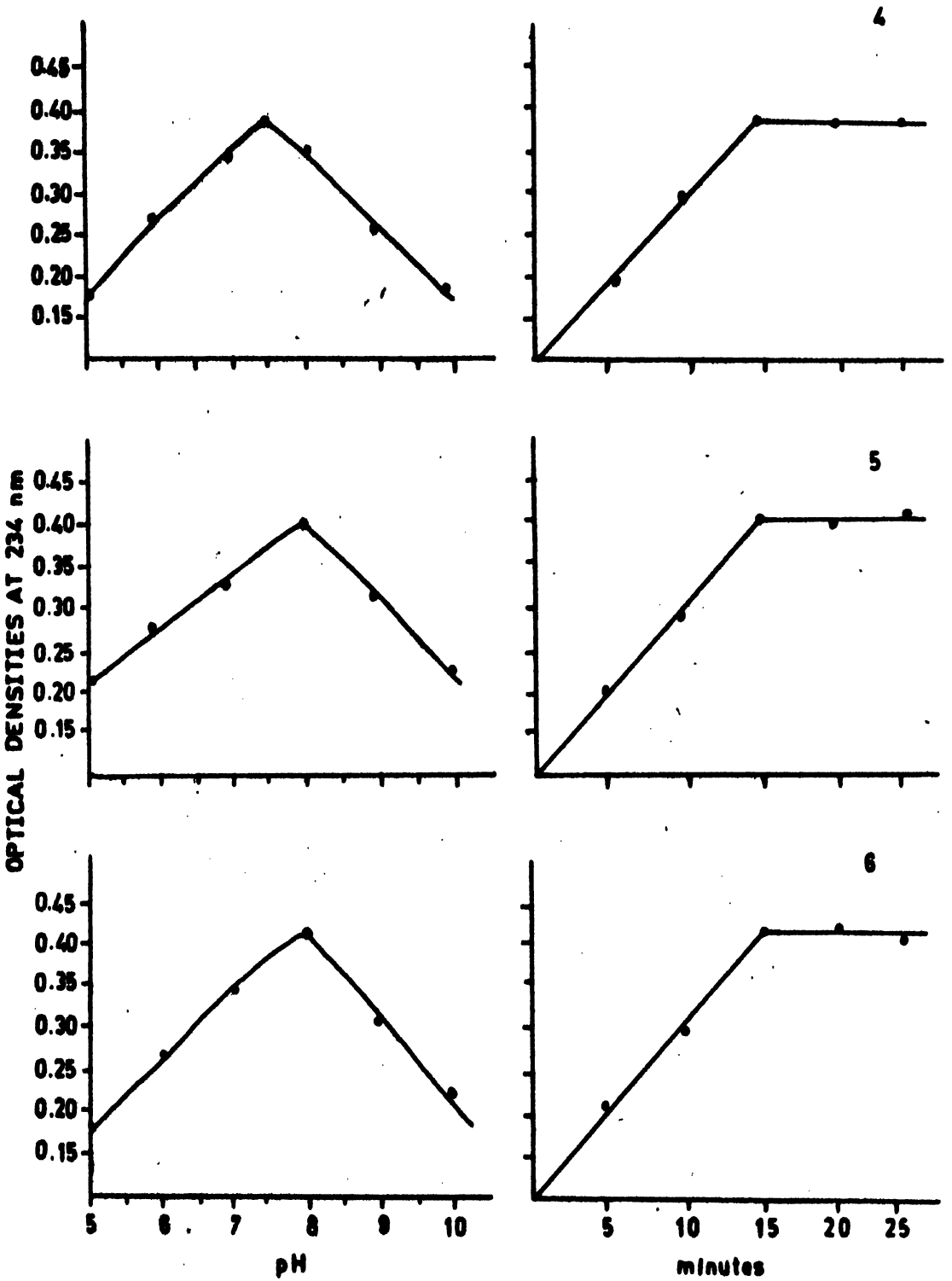

4
5
6
OPTICAL DENSITIES AT 234 nm
0.45
0.40
0.35
0.30
0.25
0.20
0.15
5
6
7
8
9
10
pH
5
10
15
20
25
minutes

Figure 3 b

Fig. 3 (c) Variation in lipoxygenase activity as a function of pH and time in different rice varieties.

7. HR - 97

8. Ambemohar

9. Basmati - Warangal

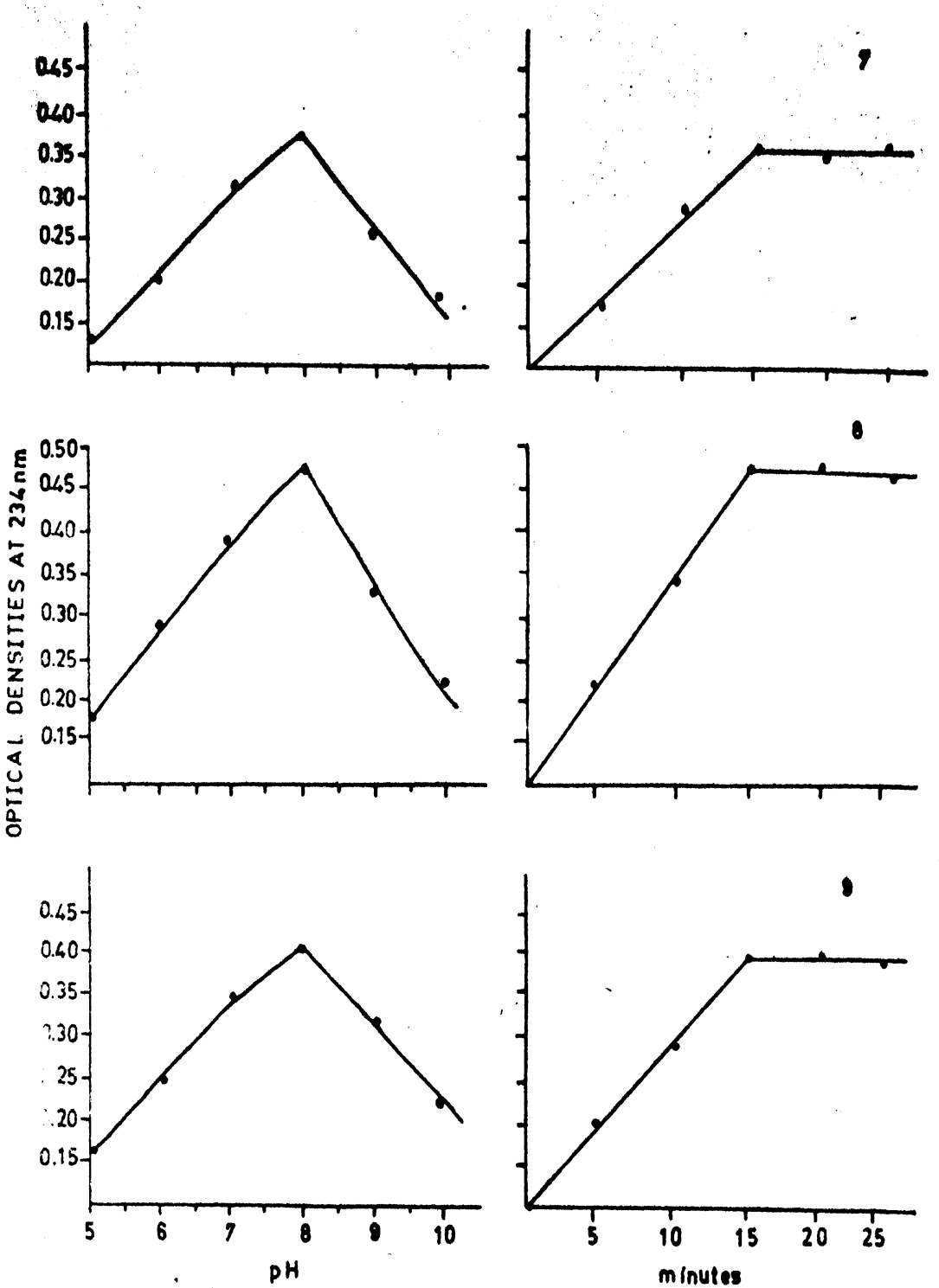

OPTICAL DENSITIES AT 234 nm
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.45
0.40
0.35
0.30
0.25
0.20
0.15
7
8
9
5
6
7
8
9
10
pH
5
10
15
20
25
minutes

Figure 3 c

Fig. 3 (d) Variation in lipoxygenase activity as a function of pH and time in different rice varieties.

10. Basmati - 427

11. IR - 8

12. Tellahamsa

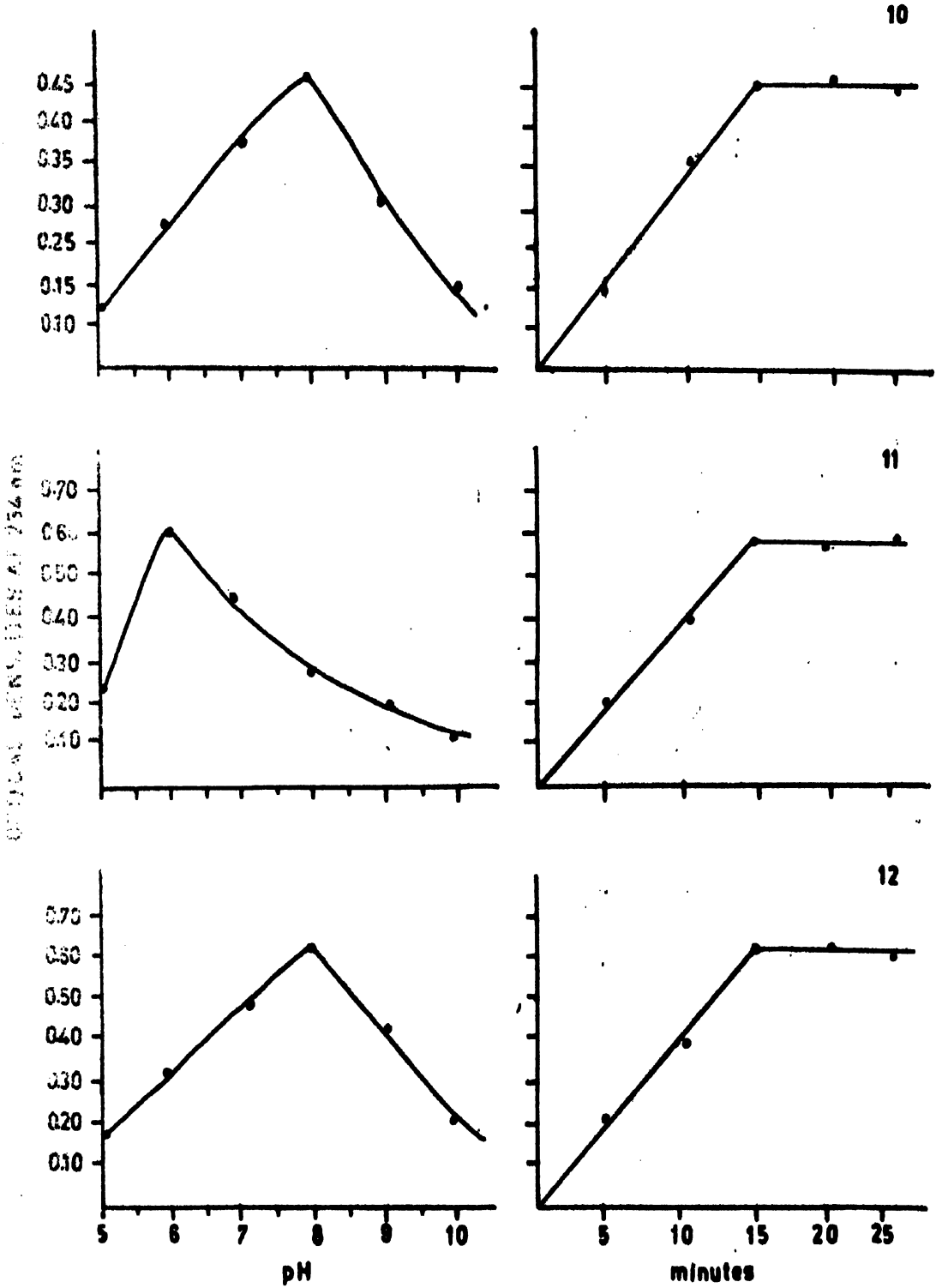

10
11
12
0.45
0.40
0.35
0.30
0.25
0.15
0.10
0.70
0.60
0.50
0.40
0.30
0.20
0.10
5
6
7
8
9
10
pH
5
10
15
20
25
minutes

Figure 3d

Similar results were obtained by Yamamoto et al(1980), in different japonica varieties where the optimal activity was found to be at pH 6.5.

These enzyme extracts were also assayed at different time intervals viz., 5,10,15 and 20 minutes at the optimal pH to study the effect of time on the activity. For all the varieties studied, there was a initial increase in the velocity, which continued upto 15 minutes and then terminated into a plateau (Fig. 3). These observations represent typical lipoxygenase patterns similar to soybean lipoxygenase (Koch et al 1958). The linear relationship is characteristic of the lipoxygenase catalyzed reaction and distinct from those nonlinear relationships usually observed for the reactions catalyzed nonspecifically by many heme proteins.

Enzyme activity:- The specific activities of different varieties are shown in the Table 6 and Fig. 4.

The scented rice varieties had lower values of specific enzyme activities when compared to non-scented varieties. The values ranged between 40 to 55 units/mg. protein for scented varieties and from 80 to 85 units/mg. protein , for non-scented varieties.

TABLE - 6

SPECIFIC ACTIVITY OF LIPOXYGENASE IN DIFFERENT RICE VARIETIES

Sl. No.	Variety	Activity (units)	Enzyme protein (mg/ml)	Specific activity (units/mg protein)
1.	Randhunipagalu	410	7.8	52
2.	Godavari Isuka	370	7.6	48
3.	Type - 3	390	8.1	48
4.	Pokura	380	7.9	47
5.	Improved Sabarmati	390	8.1	48
6.	NP 119	410	8.1	50
7.	HR 97	360	8.8	40
8.	Ambemohar	470	8.9	53
9.	Basmati Warangal	400	8.4	47
10.	Basmati 427	450	8.5	52
11.	IR 8	559	6.9	81
12.	Tellahamsa	603	7.1	85

Fig. 4. Specific activity of lipoxygenase enzyme in different rice varieties.

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3
4. Pokura
5. Improved Sabarmati
6. NP - 119
7. HR - 97
8. Ambemohar
9. Basmati - Warangal
10. Basmati - 427
11. IR - 8
12. Tellahamsa

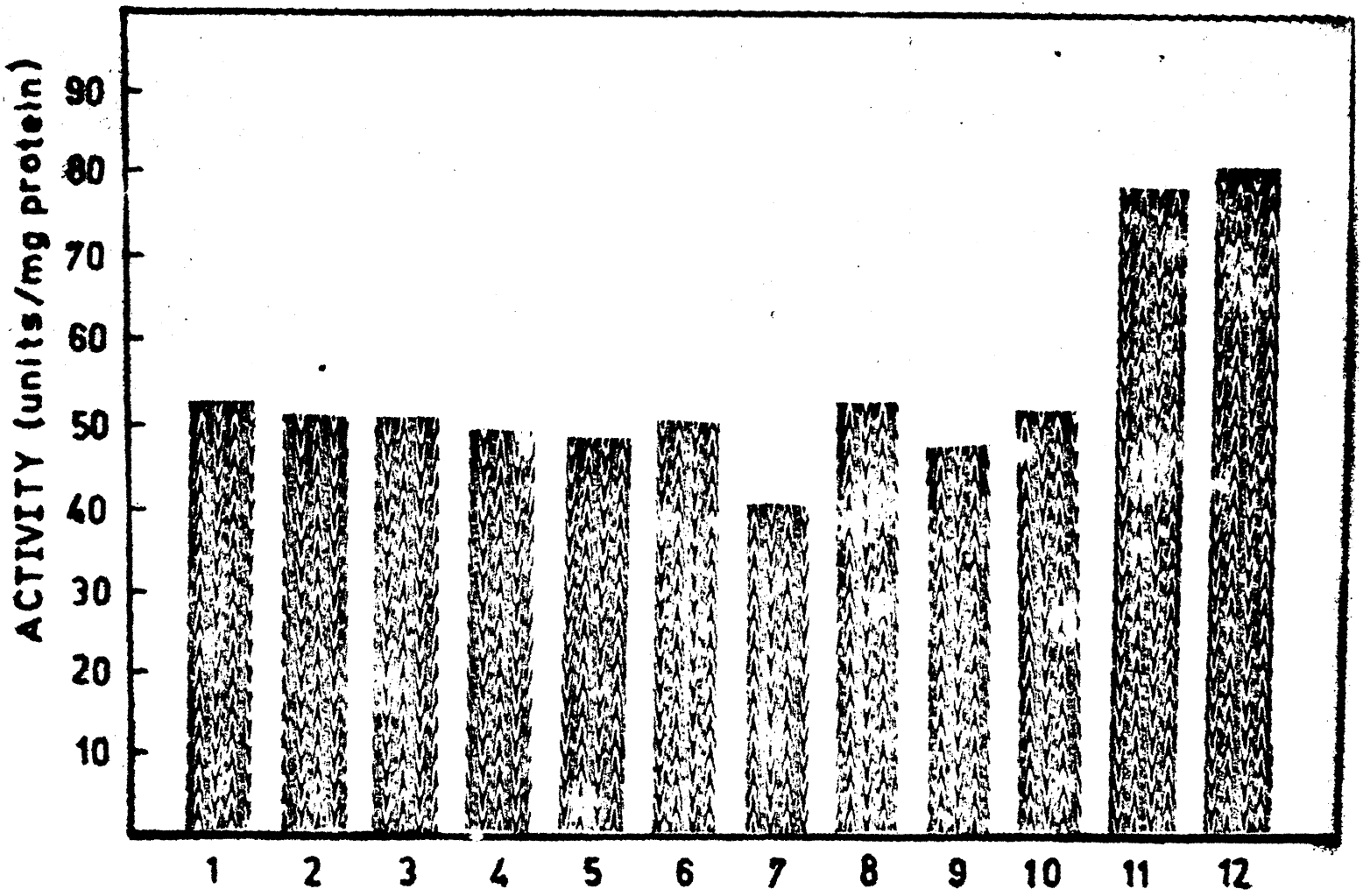

ACTIVITY (units/mg protein)
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12

Figure 4

IV. Volatile flavor component studies:

In rice, not much is known about the volatile flavor components. The differences between the composition of the volatile flavor components of scented and non-scented rices are also not clearly known. In the present study volatile flavor components were studied both quantitatively and by GLC to know the differences between scented and non-scented varieties.

Quantitative estimation of volatiles:- The quantitative estimation of volatile flavor components in different scented and non-scented varieties is given in Table 7. As can be seen from the table, all the scented rice varieties had higher mean values of volatiles than non-scented ones which was also subjected to 't' test (Table 8). The means of volatiles from varieties with known gradation of scent, viz., highly scented variety like Randhunipagalu was tested against the means of medium scented varieties like Type-3, Basmati-370 etc., and low scented varieties like Sabarmati and the means of medium scented varieties and low scented varieties were also tested by

TABLE - 7

MEAN VALUES OF VOLATILES IN DIFFERENT RICE VARIETIES

Sl. No.	Variety	Nature of aroma	Mean ± S.E. (mg/kg.of cooked rice)
1.	Randhunipagalu	Highly scented	33.80 ± 1.91
2.	Basmati 370	Medium scented	23.23 ± 0.93
3.	Likitimachi	-do-	22.62 ± 1.56
4.	Basmati local	-do-	21.74 ± 1.35
5.	Type 3	-do-	21.56 ± 1.60
6.	Godavari Isuka	-do-	20.68 ± 1.43
7.	Basmati sufaid 106	Low scented	17.48 ± 0.83
8.	Sabarmati	-do-	16.86 ± 0.92
9.	Jaya	Non scented	10.43 ± 0.71
10.	Tellahamsa	-do-	12.36 ± 0.97

TABLE - 8

COMPARISION OF MEAN VOLATILE VALUES AMONG SCENTED AND NON-SCENTED RICE VARIETIES

Sl. No.	V a r i e t i e s	't' value
1.	Randhunipagalu vs. Jaya	19.97*
2.	Randhunipagalu vs. Tellahamsa	17.35*
3.	Type 3 vs. Jaya	11.02*
4.	Type 3 vs. Tellahamsa	8.52*
5.	Basmati 370 vs. Jaya	18.82*
6.	Basmati 370 vs. Tellahamsa	13.94*
7.	Godavari Isuka vs. Jaya	11.14*
8.	Godavari Isuka vs. Tellahamsa	8.40*
9.	Likitimachi vs. Jaya	12.44*
10.	Likitimachi vs. Tellahamsa	9.16*
11.	Basmati local vs. Jaya	14.50*
12.	Basmati local vs. Tellahamsa	10.19*
13.	Basmati sufaid 106 vs. Jaya	17.80*
14.	Basmati sufaid 106 vs. Tellahamsa	9,43*
15.	Sabarmati vs. Jaya	14.29*
16.	Sabarmati vs. Tellahamsa	7.62*

't' values for 2 d.f. at 0.1 and 0.05 level of significance are 2.92 and 4.30 respectively.

* Significant,

TABLE - 9

COMPARISION OF VOLATILES AMONG HIGH, MEDIUM AND LOW SCENTED RICE VARIETIES

Sl. No.	Varieties	't' value
1.	Randhunipagalu vs. Type 3	5.94*
2.	Randhunipagalu vs. Basmati 370	7.09*
3.	Randhunipagalu vs. Godavari Isuka	6.94*
4.	Randhunipagalu vs. Likitimachi	5.53*
5.	Randhunipagalu vs. Basmati local	6.63*
6.	Randhunipagalu vs. Sabarmati	11.37*
7.	Randhunipagalu vs. Basmati sufaid 106	11.33*
8.	Type 3 vs. Sabarmati	3.81*
9.	Type 3 vs. Basmati sufaid 106	4.15*
10.	Basmati 370 vs. Sabarmati	11.37*
11.	Basmati 370 vs. Basmati sufaid 106	9.53*
12.	Godavari Isuka vs. Basmati sufaid 106	3.55*
13.	Godavari Isuka vs. Sabarmati	3.98*
14.	Likitimachi vs. Basmati sufaid 106	4.99*
15.	Likitimachi vs. Sabarmati	5.28*
16.	Basmati local vs. Basmati sufaid 106	5.13*
17.	Basmati local vs. Sabarmati	5.55*

't' value for 2 d.f. at 0.1 level of significance: 2.92.

*** Significant.**

Fig. 5. Profiles of volatiles in some rice varieties.

1. Randhunipagalu
2. Basmati - 370
3. Likitimachi
4. Basmati - local
5. Type - 3
6. Godavari Isuka
7. Basmati sufaid - 106
8. Sabarmati
9. Jaya
10. Tellahamsa

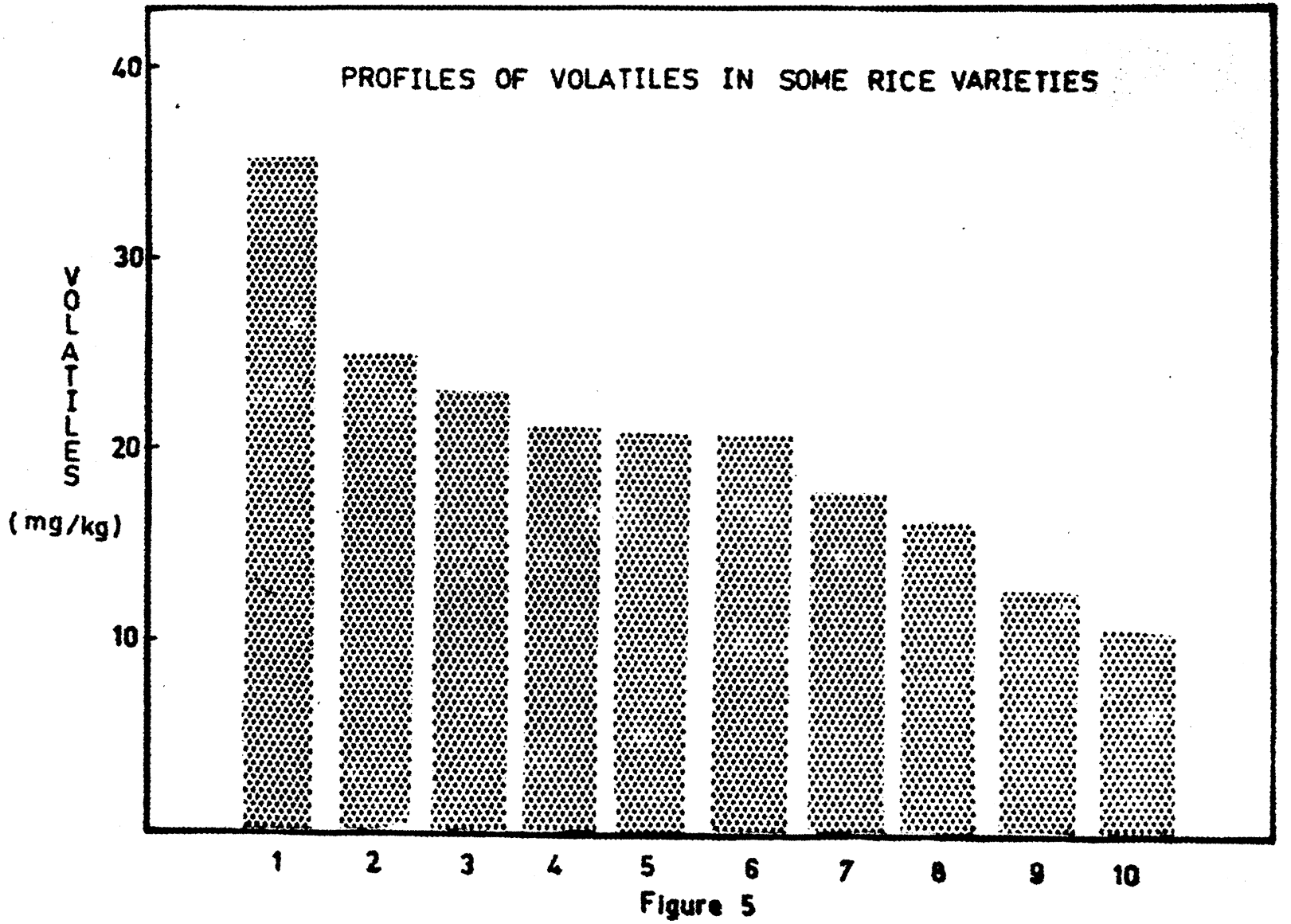

PROFILES OF VOLATILES IN SOME RICE VARIETIES
VOLATILES (mg/kg)
40
30
20
10
1
2
3
4
5
6
7
8
9
10

Figure 5

't' test (Table 9). The profiles of volatiles are shown in Fig. 5.

GLC studies with volatiles:- The concentrates of volatiles were studied by GLC to know the composition. The concentrates of volatiles from the scented rice variety 'Randhunipagalu' was subjected to GLC is in a Gas Chromatograph equipped with a device to smell the separated fractions. The pattern of GLC represented in Fig. 6. It can be seen that the Basmati flavor was coming in the initial stages of the gas chromatogram although the quantity was very low as shown by the peak.

The concentrates of volatiles from 4 scented rice varieties namely, Randhunipagalu, Godavari Isuka, Type-3 and Basmati-370 and non-scented variety Jaya were also subjected to analytical GLC. 'Randhunipagalu'(Fig. 7) contained a total of 18 peaks, Godavari Isuka'(Fig. 8) showed 17 peaks,'Type-3' (Fig. 9) exhibited 19 peaks,'Basmati-370'(Fig. 10) contained a total of 18 peaks and'Jaya'(Fig. 11) revealed a total of 18 peaks.

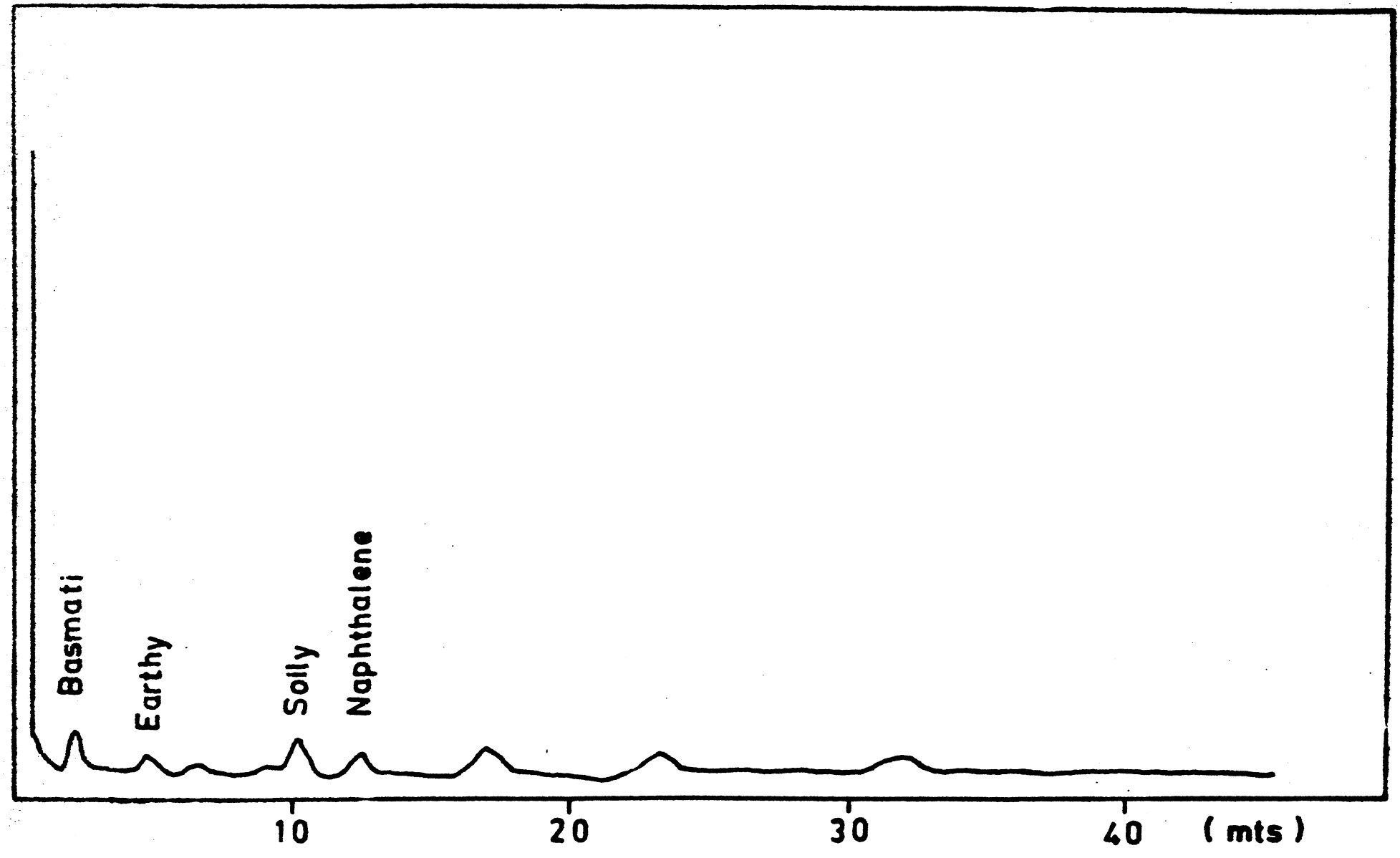


Fig.6. GAS CHROMATOGRAM OF THE AROMA CONCENTRATE WITH FLAVOR COMMENTS. var. RANDHUNIPAGALU.

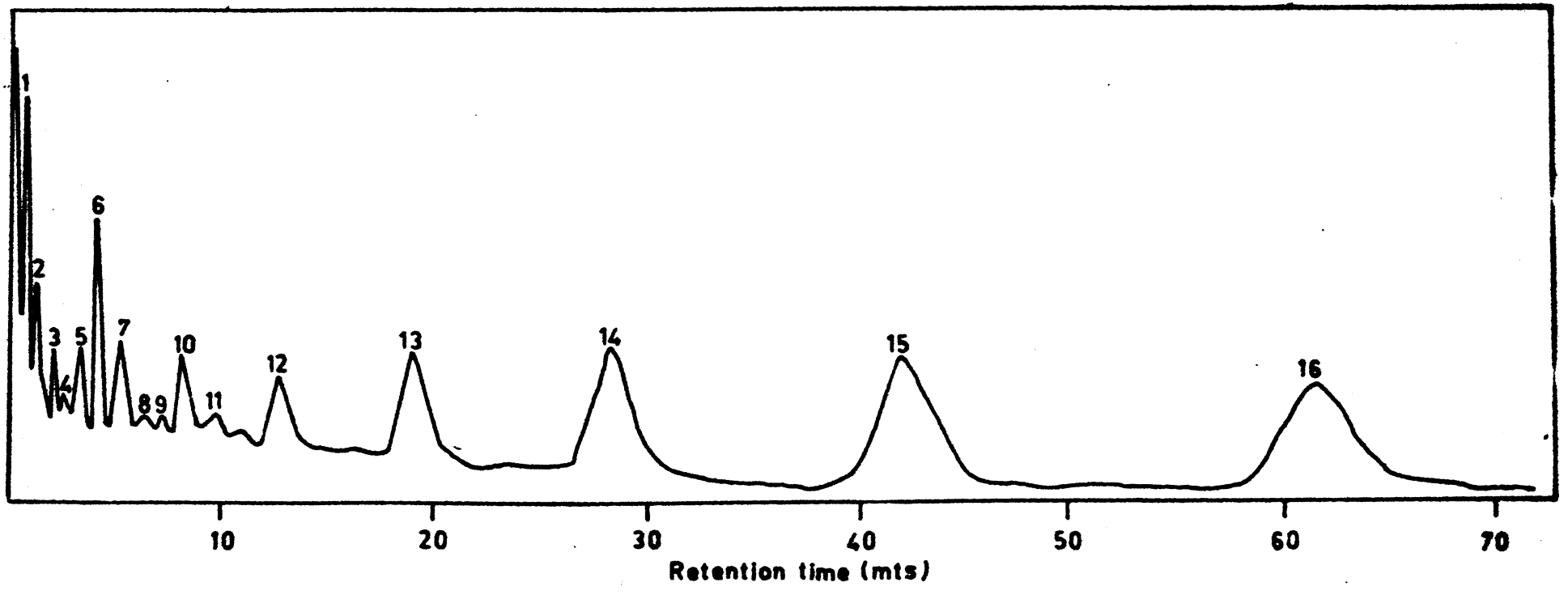


Fig.7. GAS CHROMATOGRAM OF THE AROMA CONCENTRATE . var. RANDHUNIPAGALU.

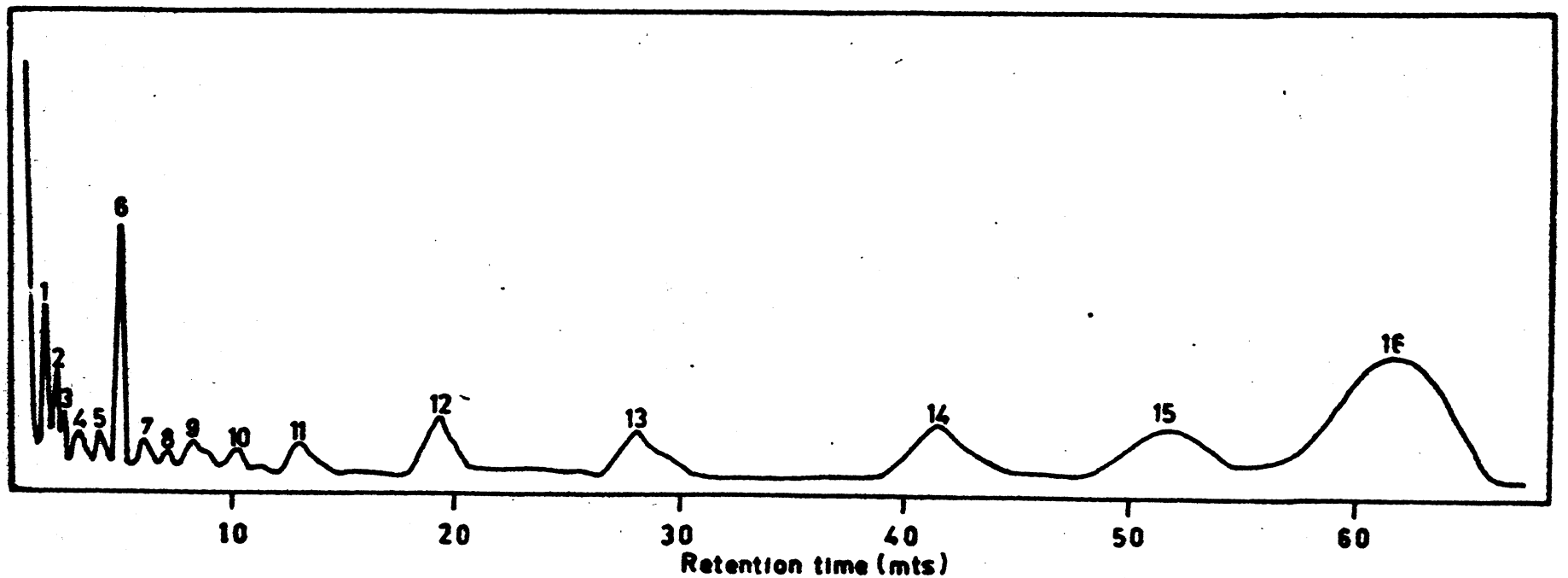


Fig.8GAS CHROMATOGRAM OF THE AROMA CONCENTRATE .var. GODAVARI ISUKA.

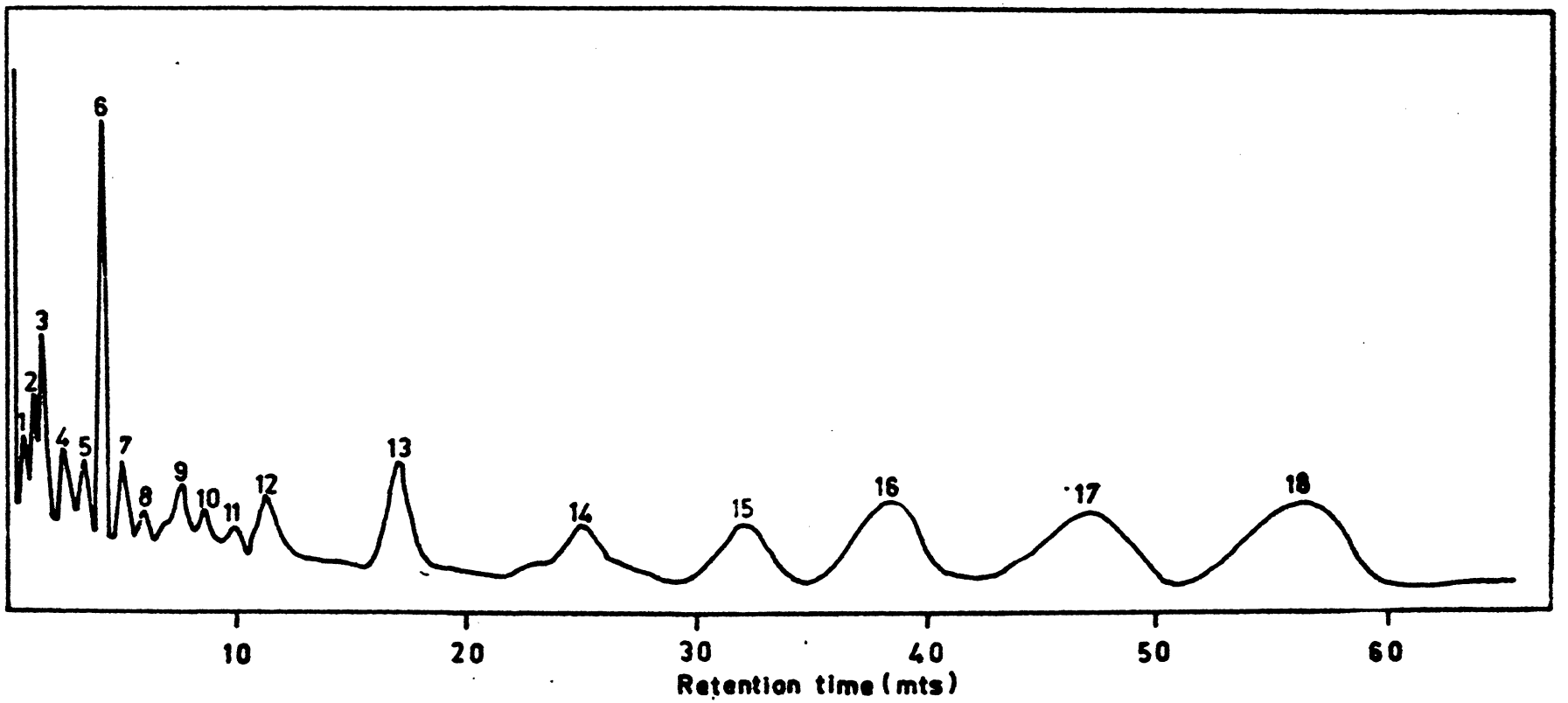


Fig.9. GAS CHROMATOGRAM OF THE AROMA CONCENTRATE. ver. TYPE-3.

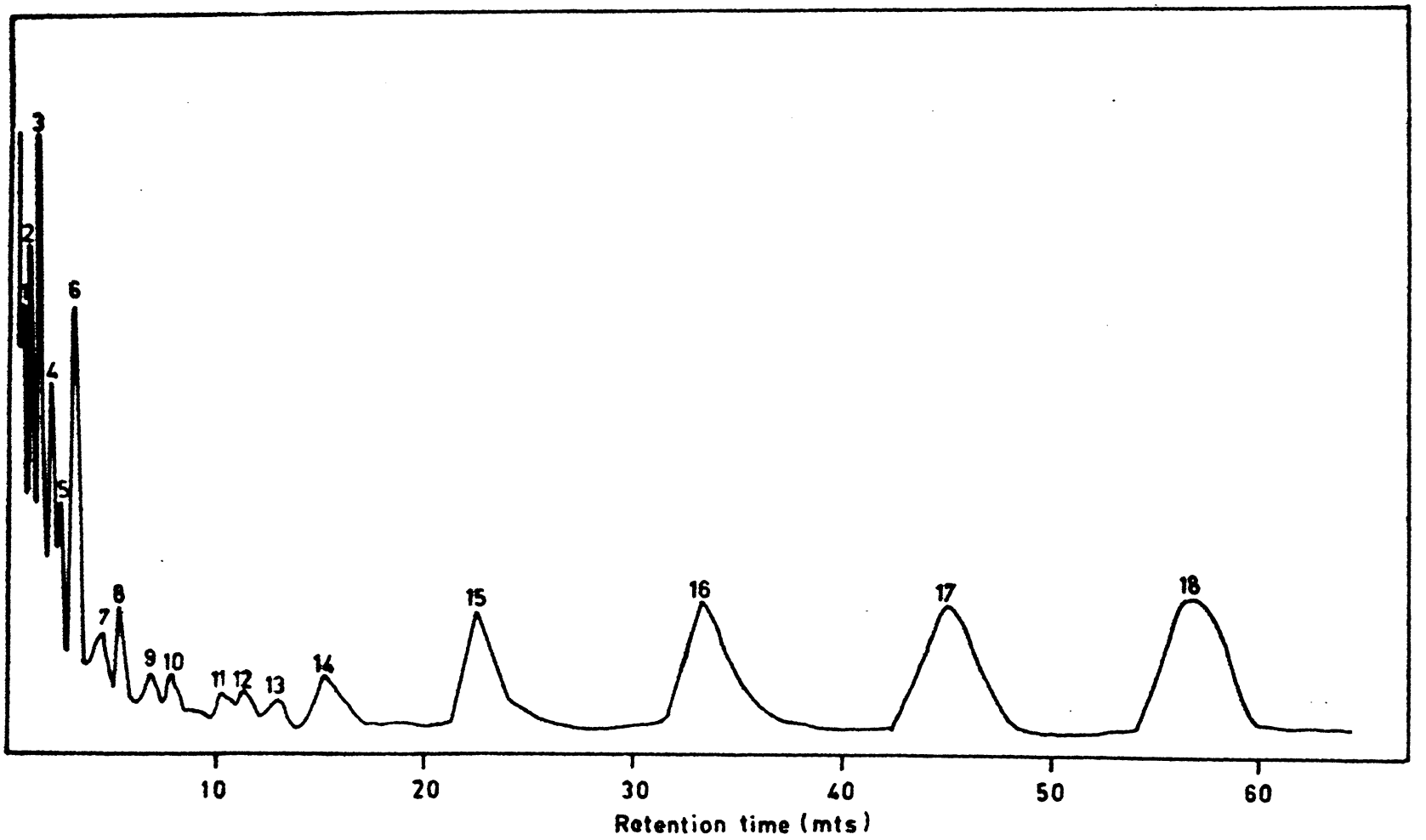


Fig.10. GAS CHROMATOGRAM OF THE AROMA CONCENTRATE. var. BASMATI - 370.

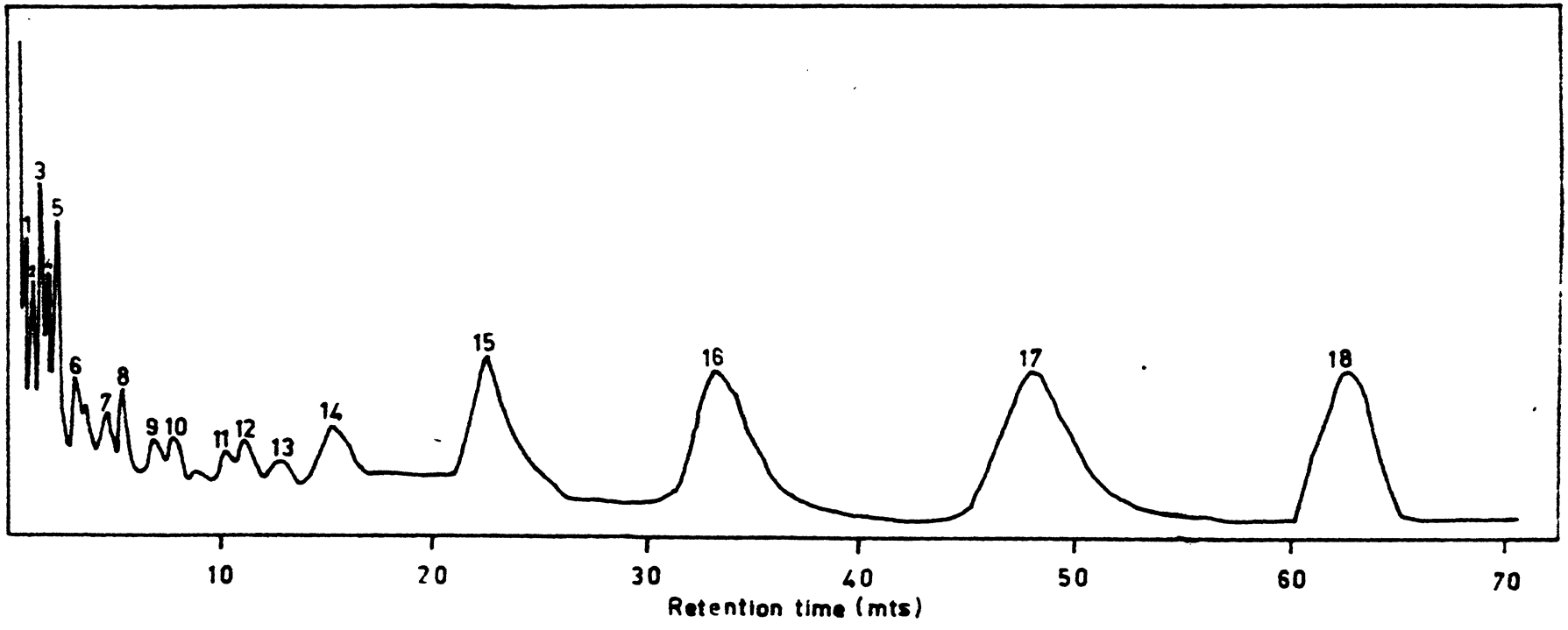


Fig.11. GAS CHROMATOGRAM OF THE AROMA CONCENTRATE.var.JAYA

V. Lipids and Fatty acid composition studies:

Lipids contribute in the production of 'aroma' by acting as precursors to volatile flavor components. In the present study, qualitative studies of lipids and fatty acid composition was studied in different scented and non-scented varieties, to see the differences among the varieties.

TLC of lipids:-

The extracted lipid samples from twenty scented and two non-scented varieties, was subjected to TLC. The separation pattern is shown in Fig. 12. The pattern of separation from the origin was, phospholipids and monoglycerides, diglycerides, free cholesterol, free fatty acid, tryglycerides and cholesterol esters. There were no class differences among the varieties. The intensities of some of the spots such as 'free fatty acid' varied in certain varieties.

Fatty acid composition:

Fatty acid composition was studied in twenty scented and two non-scented rice varieties. The typical separation of the fatty acid methyl esters

Fig. 12 (a) TLC separation of lipid from different rice varieties.

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3
4. Basmati - 370
5. NP - 119
6. Habganjbora II
7. HR - 97
8. Badshabhog
9. Basmati Sufaid - 106
10. Basmati - 140
11. Basmati - Warangal.

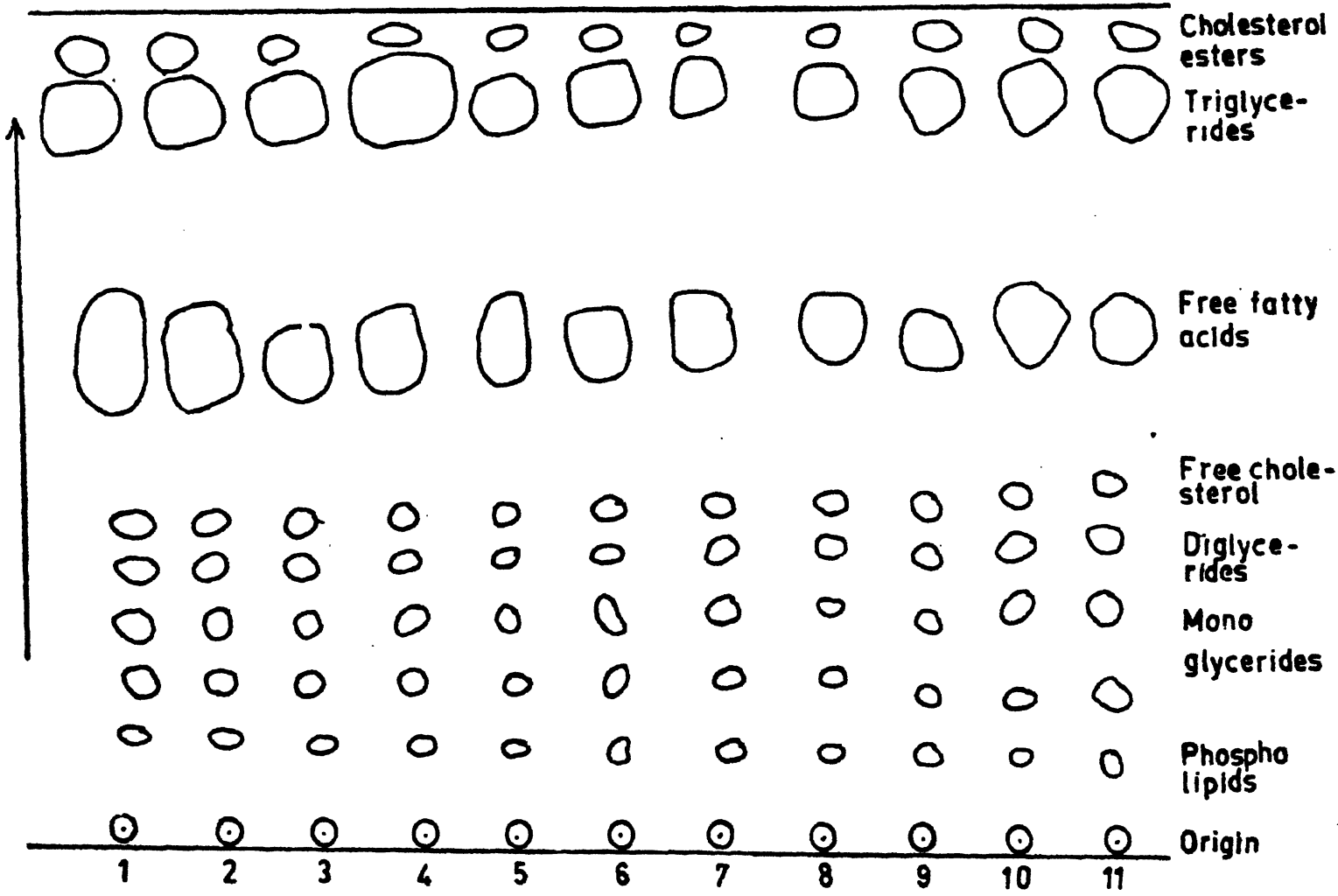

Cholesterol esters
Triglyce-rides
Free fatty acids
Free chole-sterol
Diglyce-rides
Mono glycerides
Phospho lipids
Origin
1
2
3
4
5
6
7
8
9
10
11

Figure 12 a

Fig. 12 (b) TLC separation of lipid from different rice varieties.

12. Pokura
13. Chandanchur
14. Basmati - 427
15. Ambemohar
16. Kalanamak
17. Likitimachi
18. Improved Sabarmati
19. Desi Basmati
20. Gopalbhog
21. IR - 8
22. Tellahamsa

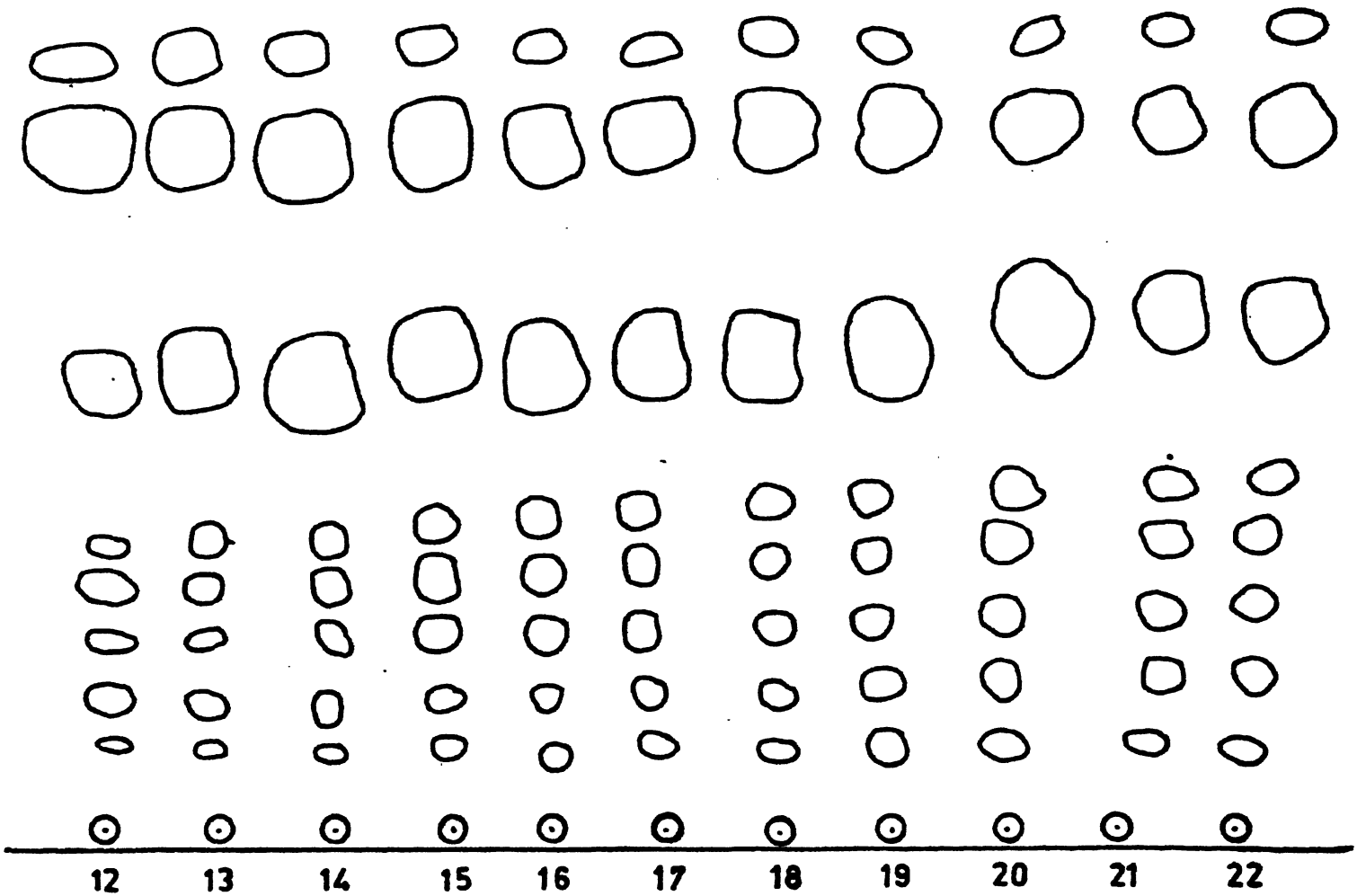


Figure 12 b

is shown in Figure.13. All together eight fatty acids, namely, myristic (C 14:0), palmitic (C 16:0), palmitoleic (C 16:1), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), linolenic (C 18:3) and arachidic (C 20:0) were present in all the varieties. Of these palmitic, oleic and linoleic were the principal components, the other being present in smaller quantities.

The fatty acid composition of different varieties is shown in Table 10. Although there were no qualitative differences in fatty acid composition pattern among scented and non-scented rice varieties, there were quantitative differences with respect to certain acids. In all the scented rice varieties, the quantity of oleic acid was higher when compared to non-scented rice varieties. It varied from 44.17% to 49.26% in the scented rice varieties, while its percentage in the non-scented rice varieties was 37.3% and 38.6%. The quantity of linoleic acid ranged from 19.07 to 25.54 among scented varieties, whereas the non-scented varieties had the values of 37.30% and 38.60%. This shows that scented varieties have lower quantities of linoleic acid than non-scented varieties.

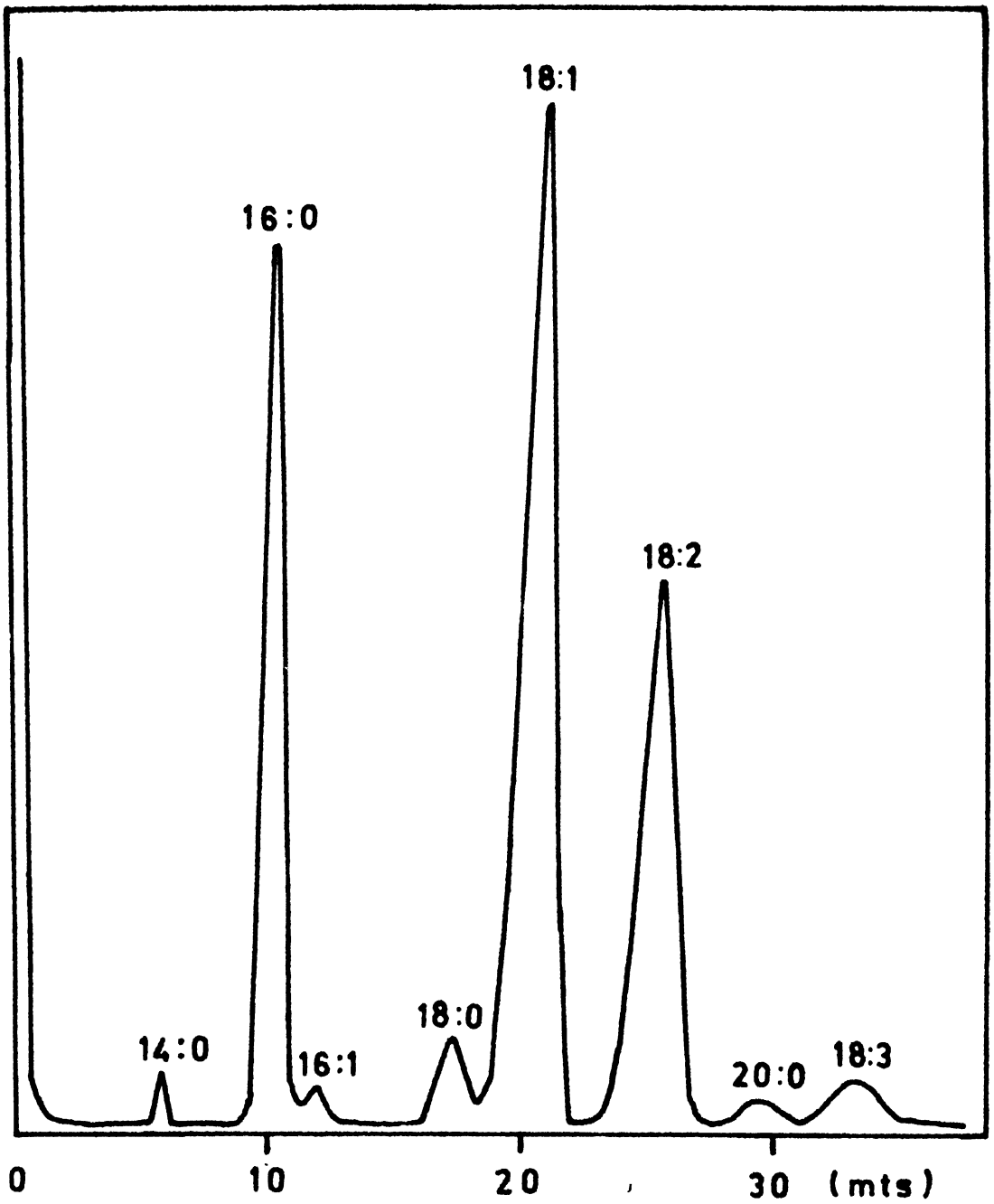


Fig.13. TYPICAL SEPARATION OF FATTY ACID METHYL ESTERS

TABLE - 10

FATTY ACID COMPOSITION OF DIFFERENT RICE VARIETIES

Sl. No.	Variety	14:0 Myristic	16:0 Palmitic	16:1 Palmitoleic	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic
1.	Randhunipagalu	0.91	19.29	0.14	2.36	46.51	25.30	2.16	1.49
2.	Godavari Isuka	0.60	22.72	0.53	2.39	45.86	25.54	1.88	0.60
3.	Type 3	0.33	21.15	0.32	2.00	47.83	26.25	1.82	0.57
4.	Basmati 370	0.56	25.18	0.39	2.17	44.17	25.00	1.96	0.73
5.	NP 119	0.72	30.07	0.42	1.08	49.26	19.93	1.08	0.47
6.	Habganjbora II	1.60	27.28	0.44	0.75	48.23	20.47	1.18	0.83
7.	HR 97	0.83	27.85	0.58	0.88	46.69	24.21	0.10	0.13
8.	Badshabhog	0.95	25.71	0.68	1.12	47.38	22.89	0.78	0.76
9.	Basmati Sufaid 106	1.47	26.27	0.17	1.30	45.01	21.89	1.74	1.30
10.	Basmati 140	0.96	29.07	0.29	1.42	47.72	21.16	0.48	0.69
11.	Basmati Warangal	0.59	28.91	1.42	1.39	47.57	20.08	0.71	0.72
12.	Pokura	0.58	29.60	0.23	1.67	48.87	19.89	0.53	0.69
13.	Chandanchur	0.99	30.34	0.33	0.93	46.43	19.07	0.66	2.15
14.	Basmati 427	0.32	23.62	0.49	0.87	48.35	25.42	0.45	0.77
15.	Ambemohar	0.85	24.40	0.75	1.22	47.50	21.72	1.10	1.50
16.	Kalanamak	1.20	28.20	0.92	0.95	46.35	21.05	0.85	0.45
17.	Likitimachi	0.78	27.50	1.05	0.78	47.36	21.48	1.05	0.40
18.	Improved Sabarmati	1.05	21.62	0.87	0.67	48.72	25.32	0.72	0.69
19.	Desi Basmati	0.69	23.09	0.49	1.05	47.92	25.62	1.03	0.54
20.	Gopalbhog	0.57	22.48	0.83	0.88	47.78	23.98	2.36	0.96
21.	IR 8	0.33	22.62	0.18	1.38	38.60	30.64	1.52	0.55
22.	Tellahamsa	0.29	21.98	0.45	1.74	37.30	33.81	1.64	0.76

VI. Amino acid composition studies:

Amino acid composition was studied in twelve scented varieties, namely Randhunipagalu, Godavari Isuka, Type-3, Basmati-370, Seetabhog, Likitimachi, Kanakjeer, Ambemohar, Bindli, SO-19, Basmati sufaid 100, Basmati 93 R and one non-scented variety Tellahamsa. The composition is shown in Table 11. There was a wide variation for most of the amino acids among the different varieties. The values for different amino acids ranged considerably: aspartic acid (4.19 gm/ 100 gm protein to 8.79 gm/100 gm protein), threonine (2.31 to 3.66), serine (2.60 to 5.16), glutamic acid (11.78 to 16.52), proline (2.06 to 4.50), glycine (2.48 to 6.65), alanine (2.94 to 5.33), valine (3.28 to 5.33), methionine (1.82 to 3.13), isoleucine (1.82 to 3.40), leucine (4.02 to 8.95), tyrosine (1.47 to 4.23), phenyl alanine (3.56 to 5.52), lysine (2.32 to 4.86), histidine (1.21 to 2.70), argenine (4.91 to 10.85). Protein percentages varied from 7.08 to 11.36%.

The highest value for aspartic acid was found in Randhunipagalu (8.79), for threonine in Type-3 (3.66), for serine and glutamic and in Randhunipagalu

TABLE - 11

AMINO ACID COMPOSITION OF DIFFERENT RICE VARIETIES
(gm/100 gm protein)

Amino acid	Varieties												
	Randhunipagalu	Godavari Isuka	Type 3	Basmati 370	Seetabhog	Likitimachi	Kanakjeer	Ambemohar	Bindli	SO 19	Basmati Sufaid 100	Basmati 23 R	Tellahamsa
Aspartic acid	8.79	5.82	7.81	6.91	6.19	7.79	7.06	7.99	7.87	8.19	6.69	6.82	6.36
Threonine	3.46	2.73	3.66	3.34	2.31	3.22	2.84	3.28	3.53	3.47	3.29	3.01	2.58
Serine	5.16	4.06	5.44	4.71	2.61	3.96	3.71	4.58	4.52	4.83	4.29	3.99	3.97
Glutamic acid	16.52	12.65	13.36	11.98	11.78	12.14	12.73	13.99	13.62	14.48	12.31	11.97	13.15
Proline	3.65	3.97	4.50	3.97	3.37	3.52	3.89	4.03	3.86	4.04	3.73	3.69	2.06
Glycine	4.97	3.96	6.65	4.76	2.48	3.56	3.29	3.79	3.72	4.51	4.07	3.46	3.43
Alanine	4.90	3.94	5.33	4.60	3.97	4.34	4.79	4.86	4.46	5.85	5.66	4.03	2.73
Valine	5.21	3.53	5.33	5.29	3.64	3.69	3.28	3.36	3.77	4.49	3.41	3.64	4.03
Methionine	2.63	2.21	3.13	2.67	1.92	2.17	2.28	2.53	2.26	2.77	2.01	2.07	1.82
Isoleucine	2.58	2.51	3.40	2.79	1.82	2.64	2.76	2.83	2.82	2.89	2.97	2.45	2.05
Leucine	8.95	5.81	7.79	6.66	4.02	6.35	5.95	6.89	6.57	6.61	6.60	5.78	7.00
Tyrosine	2.77	2.69	4.23	3.13	1.55	1.51	1.54	1.61	1.54	2.34	2.56	1.47	1.84
Phynylalanine	4.97	4.31	5.52	4.41	3.56	3.65	4.15	4.21	4.01	5.06	4.33	3.64	4.08
Lysine	3.66	2.87	4.86	3.22	2.32	3.92	3.16	3.87	3.87	3.83	4.09	3.55	2.85
Histidine	2.03	1.52	2.70	1.92	1.21	1.95	1.81	1.87	2.27	1.86	1.76	1.84	1.75
Arginine	8.56	6.11	10.85	7.91	7.96	9.14	6.62	8.54	9.74	9.64	8.36	7.46	6.94
Protein (%)	7.08	8.09	11.36	11.22	9.80	9.23	10.18	7.03	10.89	10.18	8.36	9.95	10.20

(5.16 and 16.52), Type-3 had the highest values for proline, glycine, alanine, valine, methionine, isoleucine, tyrosine, phenyl alanine, lysine, histidine, arginine (4.50, 6.65, 5.33, 5.33, 3.13, 3.40, 4.23, 5.52, 4.86, 10.85 respectively). Proline, which is known to be the precursor for certain volatile flavor components (Yazima et al, 1979), was present in more quantity in the scented rice varieties. It varied from 3.89 to 4.50 gm/100 gm protein in the scented rice varieties, while it was only 2.06 in the non-scented rice variety. The quantity of alanine, which is also a precursor for certain volatile flavor components, was more in the scented varieties than non-scented ones. It varied from 3.94 to 5.85 as against 2.73 of the non-scented variety (Fig. 14).

Essential Amino acid pattern in different varieties:

The essential amino acids, lysine, methionine, threonine, leucine, isoleucine, valine and phenyl alanine composition in different rice varieties were compared with the recommended standards of FAO (Food and Agricultural Organisation). The pattern of these amino acids is represented in Fig. 15.

Fig. 14. Proline and alanine composition in different rice varieties.

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3
4. Basmati - 370
5. Seetabhog
6. Likitimachi
7. Kanakjeer
8. Ambemohar
9. Bindli
10. SO - 19
11. Basmati Sufaid - 100
12. Basmati - 93 R
13. Tellahamsa.

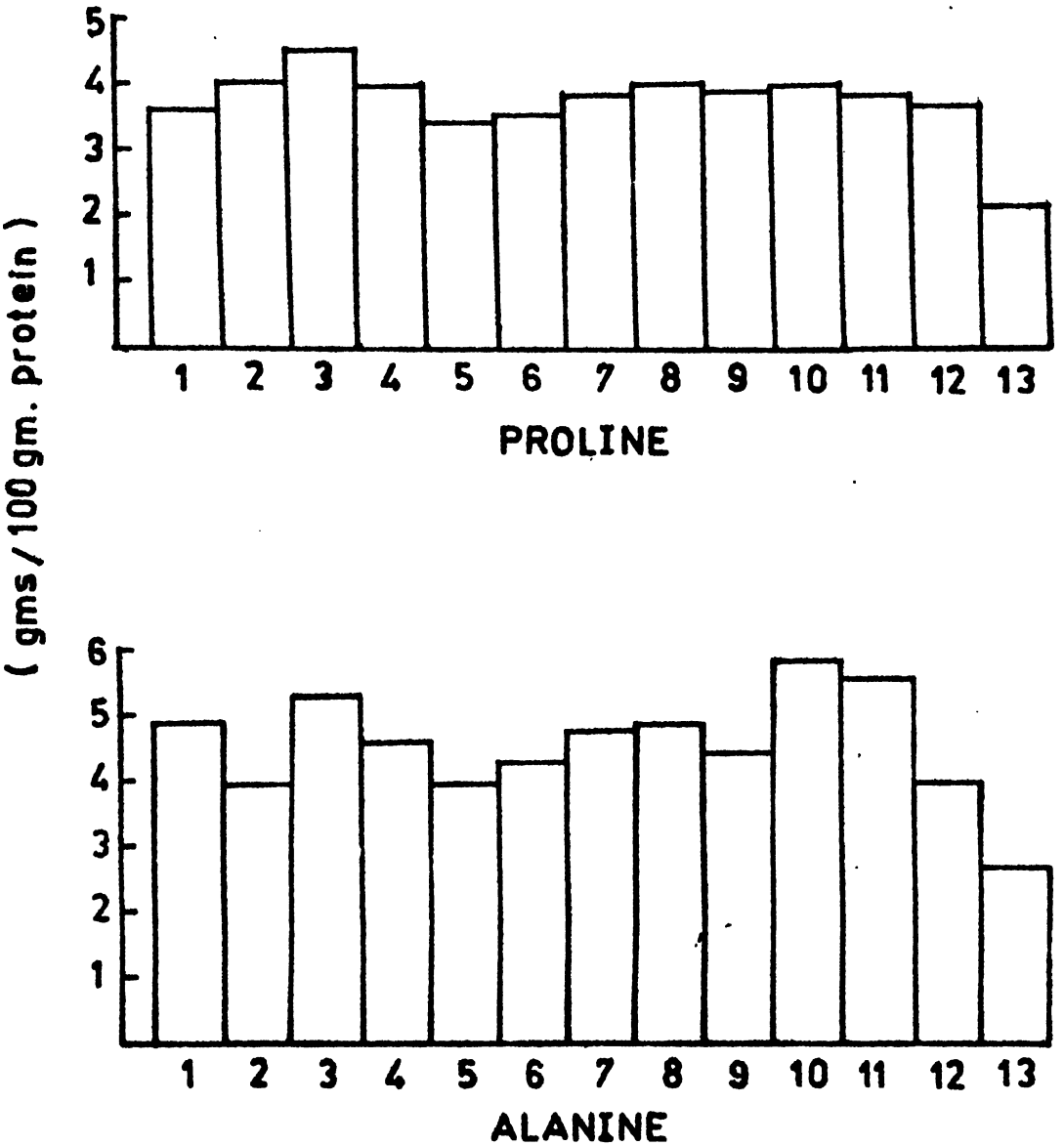

(gms / 100 gm. protein)
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10 11 12 13
PROLINE
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10 11 12 13
ALANINE

Figure 14

For lysine content, varieties Randhunipagalu, Likitimachi, Ambemohar, Bindli, SO-19, Basmati sufaid 100 had comparable values with the FAO standards. The variety Type-3 had a higher quantity of lysine than the FAO standard, and the varieties, Godavari Isuka, Basmati-370, Seetabhog, Kanakjeer, and Tellahamsa had lower quantities than the FAO standard.

For methionine content, all the scented rice varieties had higher values than the non-scented one and all the values were comparable to the FAO standard. Type-3, Basmati-370 and SO-19 had higher values among the scented varieties. Non-scented variety Tellahamsa, had lower quantity than the FAO value.

For leucine content, all the varieties with the exception of Seetabhog,had higher values than the FAO standards. Randhunipagalu had the highest value followed by Type-3.

For Isoleucine content, all the varieties had lower values when compared to FAO standards. Seetabhog and Tellahamsa had the lowest values for this amino acid.

Fig. 15. Essential amino acid profiles in different rice varieties.

1. Randhunipagalu
2. Godavari Isuka
3. Type - 3
4. Basmati - 370
5. Seetabhog
6. Likitimachi
7. Kanakjeer
8. Ambemohar
9. Bindli
10. SO - 19
11. Basmati Sufaid - 100
12. Basmati - 93 R
13. Tellahamsa
14. FAO recommended value

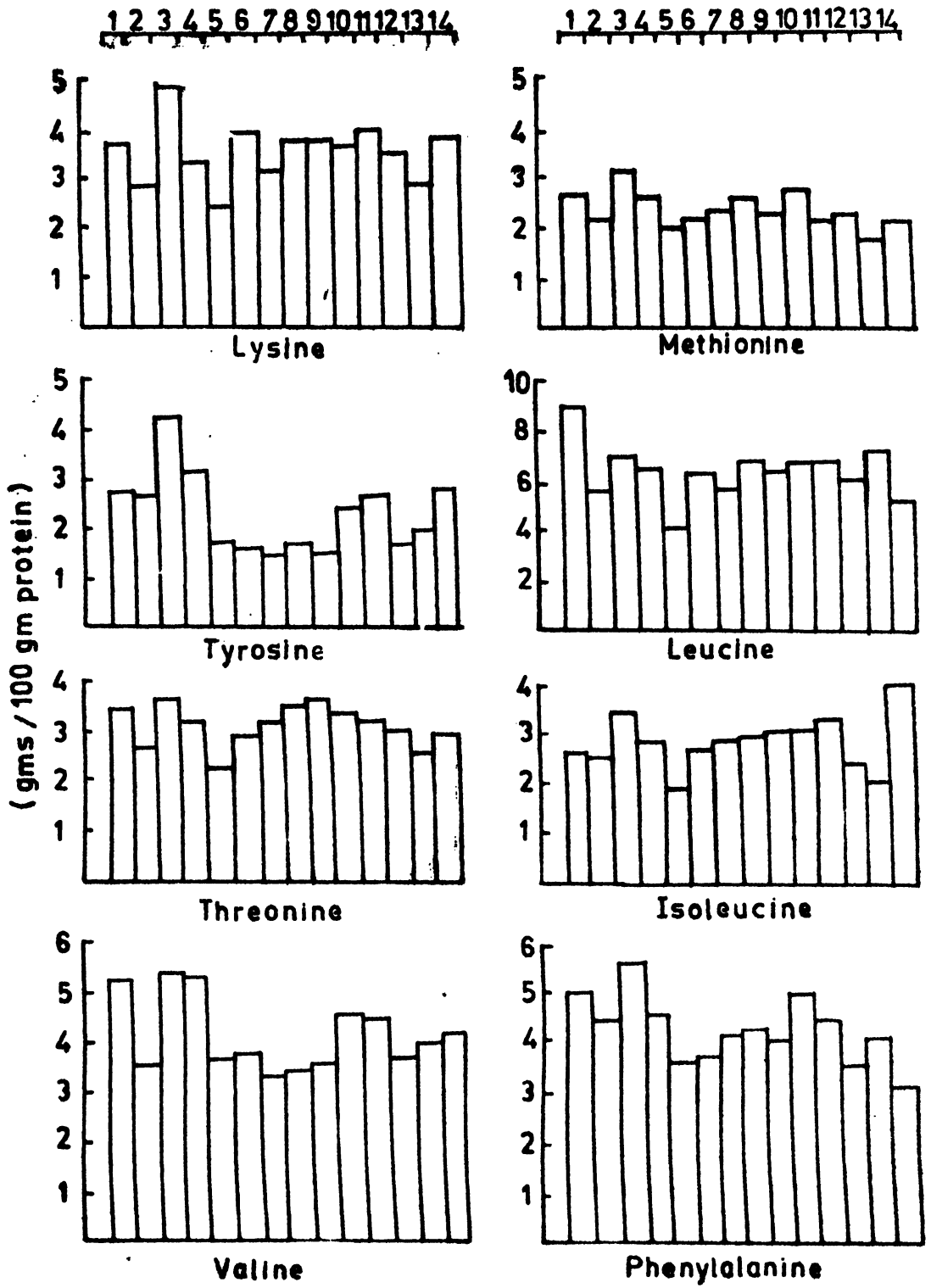

1 2 3 4 5 6 7 8 9 10 11 12 13 14
Lysine
Methionine
Tyrosine
Leucine
Threonine
Isoleucine
Valine
Phenylalanine
(gms / 100 gm protein)

Figure 15

For threonine content, all the varieties with the exception of Seetabhog and Tellahamsa, had comparable or higher values than the FAO standards. Randhunipagalu, Type-3 and Bindli exhibited highest values.

For valine content, Randhunipagalu, Type-3 and Basmati-370 had the highest values and exceeded the FAO standard. Varieties Bindli, SO-19 and Tellahamsa had comparable values and the rest of the varieties had lower values than the FAO standard.

For phenylalanine, all the varieties exceeded the FAO standard and Type-3 had the highest value, followed by Randhunipagalu and SO-19.

DISCUSSION

In view of the importance of basmati rices, attempts have been made in the past to recover high yielding, dwarf rice varieties with desirable basmati qualities, like pleasant aroma, better cooking and eating qualities (Sood 1978). In general, the released varieties failed to match with the typical basmati varieties and this may be due to lack of precise information on the genetics of aroma and the biochemical nature of the scented rices. The present investigation was mainly aimed to understand the genetics of aroma and certain biochemical characterisation studies such as esterase isoenzymes, lipoxygenese enzyme, volatile flavor components, lipids, fatty acid and amino acid composition studies.

I. Genetic Studies:

In recent years, breeding for high yielding Basmati varieties has assumed national importance in view of the export potential of these rices in Foreign markets. Varieties like Sabarmati, Improved Sabarmati etc., have been bred and released for

cultivation (Siddiq et al 1976). Although they have high yield potential, they fail to compete with the typical basmati rices in grain quality. Among the various reasons ascribed to the failure of breeders to develop high yielding varieties combining typical basmati characteristics, the most important is the lack of information with regard to the genetics of this trait in different varieties.

The F_1 plants of five crosses between scented and non-scented varieties, were all non-scented, suggesting that non-scentedness is dominant. The F_2 segregation data showed 3:1 (non-scented : scented) ratio, and X^2 and 'p' values confirmed this segregation ratio (Table 3). These observations suggest that 'aroma' in rice is inherited as a monogenic and recessive character. Earlier observations of Ramaiah (1937), Dave (1940) and Srinivasan (1941) suggested that although the scented character was monogenic, it exhibited dominance. A digenic ratio of 9 scented : 7 non-scented (Ramaiah 1937) and 15 scented : 1 non-scented (Srinivasan 1941, Dhulappanavar and Mensinkai 1969) and a trigeneric complimerary ratio of 27 : 37 was reported by Kadam and Patankar (1938) and

Nagraj et al (1975), on the contrary to the present observations. Chakrabarthy (1948) obtained a ratio of 13 : 3 (non-scented : scented) and Dhulappanavar (1976) suggested that four complimerary genes govern 'aroma' in rice. Ghose et al (1960) and Sood and Siddiq (1978), however reported that the scented trait is inherited as a monogenic and recessive character. The descrepancies of earlier workers may be due to the analysis of aroma from the seeds of F_2 plants, which are in fact F_3 seeds. Apart from this, these workers may have suffered from lack of precise scoring methods for 'aroma' (Ramaiah and Rao 1953). These workers either chewed the scented grains directly or boiled them, which might have resulted into aberrant ratios. In the present study, the scoring was done by treating the F_2 plant material to mild alkali solution and then observing the aroma (Sood and Siddiq 1978), this eliminated the errors in scoring.

These observations clearly suggest that due to monogenic recessive inheritance, the 'gene' governing basmati qualities, can be successfully exploited in breeding programmes by using as one of the donors.

II. Esterase isoenzyme studies:

In the present study, esterase isoenzyme studies, in five day old seedlings of thirty scented and six non-scented rice varieties, suggest that only three isozyme bands 6, 9 and 10 were common, where as bands like 8, 11, 12 exhibited differences among the varieties (Table 4). Mitra et al (1970) studied esterase isoenzymes of 40 cultivars of Hordem vulgare, and found that there were nine different types of zymograms, of which two were more frequent.

When the electrophoretic pattern of scented and non-scented rice varieties were compared, there were clear differences between the two groups. All the non-scented rice varieties, had two medium stained bands, which were absent in all the scented varieties. These are band 8 (Rf 0.46) and band 11 (Rf 0.63), whose activity ranged from 2.12 to 3.71 and 3.65 to 5.10 respectively (Table 5). Payne and Koszykowski (1978) in their studies on the esterasc isoenzymes of Soybean cultivars, reported that although there were no qualitative differences, quantitative differences were observed with regard to two isoesterases

and the cultivars could be identified based on these differences.

The qualitative differences between scented and non-scented rice varieties suggest that these differences could be used as a reliable parameter in characterising scented and non-scented rice varieties, since the presence or absence of a band and its intensity on the gel is a reflection of the presence of active enzyme, which is controlled by the specific gene(s).

Based on these isoenzyme studies it can be assumed that, due to the absence of specific isoenzymes (bands 8 and 11) in scented varieties, some of the esters might be accumulated only in these varieties. These accumulated esters may be responsible in the production of certain volatile flavor components and might be acting as precursors. On the contrary, in the non-scented varieties, presence of these isozymes, may result in the degradation of the particular esters and probably are not available for the synthesis of flavor compounds.

The zymograms were compared among the four categories of scented rice varieties, such as high and medium-high scented, medium scented, medium-low scented and low scented varieties.

The varieties of high and medium-high scented group exhibited similar types of electrophoretic pattern, with a few exceptions, and all exhibited a darkly stained band 12 (Rf 0.65) which was absent in all other groups. The activity of this band varied from 19.80 to 40.45 (Table 5). This group was also characterised by the absence of band 7, which was present in medium and medium-low scented groups and absent in non-scented group. Band 1 (Rf 0.06) which was present in other groups was absent in this group.

The varieties of medium scented group, displayed similar zymogram pattern. All the varieties had a darkly stained band 14 (Rf 0.75) whose activity varied from 24.36 to 40.75, beside band 1 (Rf 0.06) which was absent in high, medium-high and medium-low scented groups. Bands 9 and 10 exhibited slightly reduced activity when compared to other groups, suggesting that these bands may be associated with quantitative

gradation of scentedness. It varied from 8.97 to 15.71 for band 9 as compared to the values of 14.31 to 23.33 of other groups. The values for band 10 were 11.54 to 19.64 as compared to the values of 18.67 to 43.74 of other groups.

The varieties of medium-low scented group, evinced similar types of electrophoretic pattern. Most of them exhibited a lightly stained band 7 (Rf 0.27) which was absent in high, medium-high and low scented groups. The activity of this band varied from 0.57 to 3.51. All these varieties had a lightly stained band 13 (Rf 0.68), which was absent in high and medium-high and medium scented groups.

The electrophoretic pattern studies among different groups of scented rices, suggest that there are qualitative and quantitative differences between these groups. Differences in zymogram pattern could be utilised for characterisation and identification of the varieties of a particular group. There were qualitative differences in different groups, such as the presence of band 12 in high and medium-high groups and its absence in all other groups, the presence of band 1 in medium-scented group and its

absence in high and medium-high, and medium-low groups, which might be exploited in characterisation of the varieties. There were quantitative differences also, of the similar bands in different groups, such as the reduced activity of bands 9 and 10 in the medium scented group (Table 5). Esterase isoenzyme differences among Soybean cultivars, showed that out of five bands identified (A, B, C, D, E), the activity of bands A, B, C decreased on storage and hence they were not used. On the other hand, there was no variation in the staining intensity of band D due to storage and it exhibited staining differences which were used in characterisation of varieties (Payne and Koszykowski 1978). In the present study also, there were clear qualitative and quantitative differences among the varieties and this should help in differentiating the varieties.

As described earlier, the non-scented varieties exhibited the maximum number of bands when compared to scented varieties. Association of decrease in aroma with the increase in the number of isozyme bands was an interesting observation. In the high and medium-high groups, altogether there were 8

isozyme bands in different varieties and in medium scented group there were 9 bands. The varieties of medium-low and low scented groups exhibited 10 bands and the non-scented variety showed 11 bands. The presence or absence of a band and its intensity on the gel is a general reflection of the enzyme activity (Mitra et al 1970), and it may be assumed that by the presence of these additional isozyme bands, the specific esters may not be available for the biosynthesis of volatile flavor components. Similarly the gradual increase of number of bands may have a role in the 'reduction of aroma' in the scented varieties of different groups.

III. Lipoxygenase enzyme studies:

Quantitative studies of lipoxygenase in the seed samples of ten scented and two non-scented rice varieties, at different pH levels exhibited the optimum enzyme activity around pH 8.0 (with the exception of variety IR 8) (Fig. 3). Similar observations were recorded by Shastry and Rao (1975) in their study of lipoxygenase from rice bran, where the optimum pH was 8.5. The activity of the enzyme from the variety IR 8,

showed on optimal pH of 6, unlike the rest of the varieties. Yamamoto et al (1980), in their studies on lipoxygenase from different japonica varieties, reported an optimal pH of 6.5 and the discrepancies could be varietal and differences between japonica and indica rices. The exception of IR 8, as observed in the present study could be due to its japonica background.

An optimal pH of 5.5 was reported for potato lipoxygenase (Pinsky et al 1973) and most workers found a bell shaped curve with maximal activities between pH 7 and 8 for the soybean enzyme (Siddiqui and Tappel 1957). Surrey (1964) reported an optimal pH of 6 for soybean meal lipoxygenase, and the activity declined sharply towards the acid side whereas on the alkaline side it declined gradually upto pH 7 and levelled off at pH 8.0.

The assay of enzyme extract at different time intervals viz., 5, 10, 15 and 20 minutes, in the present study showed that there was an initial increase in the velocity which continued upto 15 minutes and then terminated into a plateau. Similar observations

were obtained by Surrey (1964) in studies on the lipoxygenase of grain flour, where there was an increase in the activity upto 13 minutes and then resulted into a plateau. These observations were typical of earlier studies and it can be concluded that the rice enzyme also belongs to the true lipoxygenase group of enzymes.

During storage of rice, one of the unfavourable changes occurring is the development of stale and objectionable flavour in the grain. Hexanal, is one of the major component attributable to the development of undesirable flavors in many plant foods. In long term stored rice kernels, hexanal is also an important flavor contributor and is presumed to arise from the decomposition of hydroperoxides of unsaturated fatty acids (Yasumatsu et al 1966). It was shown by GLC studies that there was a significant increase in the hexanal content of stored rice when compared to fresh rice. As lipoxygenase is implicated with heme proteins in the hydropheroxide production in many plant systems, it may be desirable to study this enzyme in rice, since development of stale flavor in the grain is the limiting factor for having long term

storage objectives.

In the present study, varietal differences were observed when specific activities of the enzyme were compared (Table 6). The activities ranged from 40 to 55 units per mg/protein for the scented rice varieties and from 80 to 85 units per mg/protein for the non-scented varieties IR 8 and Tellahamsa. This shows that all the scented varieties were characterised by low activities of this enzyme as compared to the high activities of the non-scented varieties. Since linoleic acid is the precursor for some of the compounds like hexanal which are responsible for the production of off-flavors during storage of rice, the linoleic acid composition of the different varieties was also compared (Table 10). Apart from the ten scented varieties used for lipoxygenase studies, ten more scented varieties were used for fatty acid composition studies, besides the two non-scented varieties. The comparison of linoleic acid content among these varieties revealed that the values ranged from 19.07 to 25.54% among scented varieties, whereas the non-scented varieties had the values of 37.30 to 38.60%, suggesting that there are clear differences

between scented and non-scented rice varieties. When the lipoxygenase activity and linoleic acid content were compared among the varieties, an interesting correlation was observed. The non-scented varieties which had the highest values for lipoxygenase activity also had highest values for linoleic acid content (Table - 12). The scented rice varieties which had lower values of lipoxygenase, had correspondingly lower values of linoleic acid also. This is a significant observation as both the linoleic acid and lipoxygenase are involved in the formation of volatile compounds which produce off-flavours in rice during storage. The scheme of degradation for linoleic acid is shown in Fig. 16. It can be seen from the figure that the formation of 11-C peroxides is remarkable and those of other 8-C and 14-C peroxides are few. Thus formed peroxides are decomposed into aldehydes by heat, light, metals and radiation etc. C_3, C_5 and C_6 aldehydes were proved to be the degradation products of linoleic acid (Hoffman 1962). Of these aldehydes, C_6 aldehyde (hexanal) was reported to be the major product. As seen earlier, these C_3, C_5 and C_6 aldehydes are reported to be present

TABLE - 12

COMPARISION OF LIPOXYGENESE ACTIVITY AND LINOLEIC ACID CONTENT IN DIFFERENT RICE VARIETIES

Sl. No.	Variety	Enzyme activity (units/mg protein)	Linoleic acid content (%)
1.	Randhunipagalu	52	25.30
2.	Godavari Isuka	48	25.54
3.	Type 3	48	26.25
4.	Pokura	47	19.89
5.	Improved Sabarmati	48	25.32
6.	NP 119	50	19.93
7.	HR 97	40	24.21
8.	Ambemohar	53	21.72
9.	Basmati Warangal	47	20.08
10.	Basmati 427	52	25.42
11.	IR 8	81	30.64
12.	Tellahamsa	85	33.81

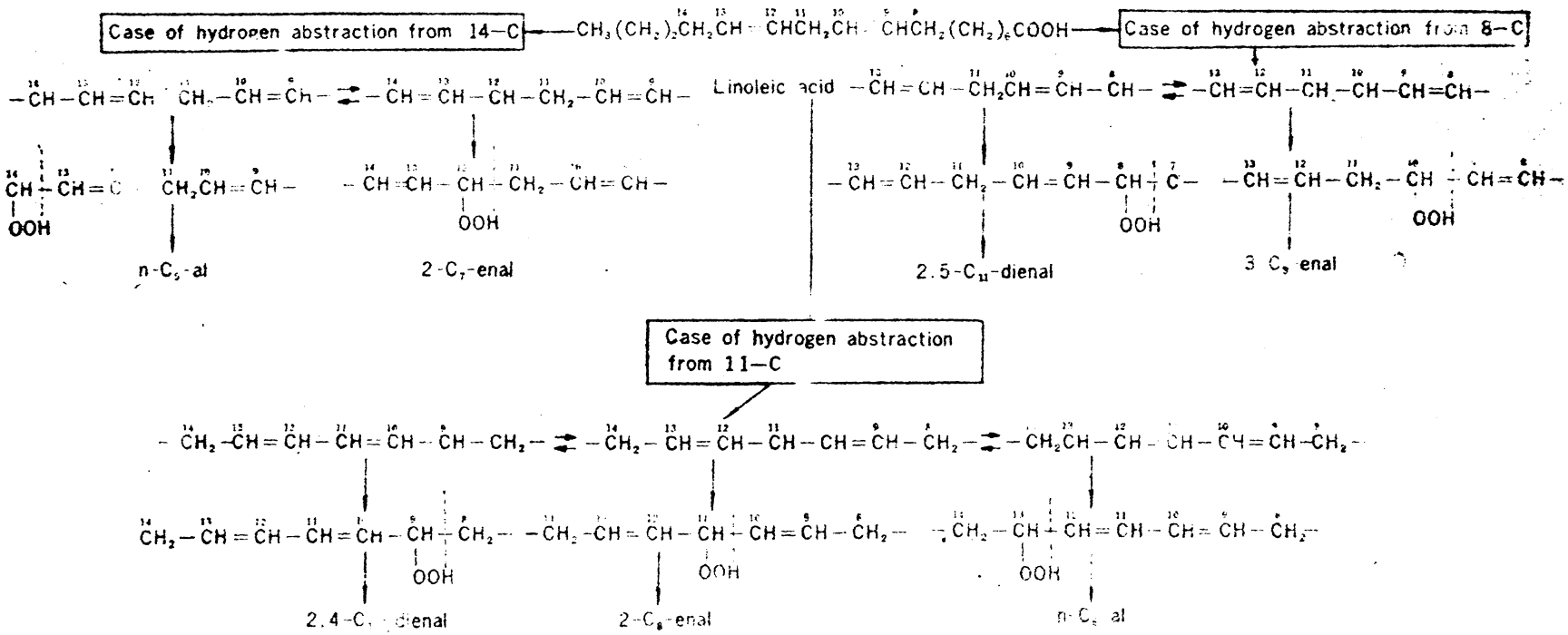


Fig.16. Scheme of Oxidation of Linoleic acid (After Chikubu 1970)

in higher concentrations when stored rice is cooked. It can be assumed that these aldehydes are formed as a result of the degradation of linoleic acid (Chikubu 1970).

Since lipoxygenase is involved in the degradation of unsaturated fatty acids such as linoleic, linolenic, it can be assumed that this enzyme plays a significant role in the liberation of off flavour in rice during storage, by catalysing the production of hydroperoxides from unsaturated fatty acids. The correlation between lipoxygenase activity and linoleic acid content also lends further evidence to this assumption.

Based on these observations, it is reasonable to assume that the scented varieties may have better storage qualities than the non-scented varieties, due to the fact that more quantity of linoleic acid coupled with high levels of lipoxygenase activity result in the production of off flavours more rapidly. Further studies on the characterisation and positional specificity of the enzyme reaction are necessary to understand the role of this enzyme in the production of off flavours in rice.

IV. Volatile flavour components:

The observations on the quantitative estimation of volatile flavour components are summarised in Table 7. The scented rice varieties had higher mean values of volatiles than the non-scented varieties and the values were statistically significant (Table 8). These observations clearly suggest that scented rice varieties have significantly higher amounts of volatile flavour components than non-scented varieties and the pleasant aroma of scented rices is due to the presence of higher quantities of volatile flavour components. This was also proved by the fact that the concentrates of volatiles from scented rice varieties exhibited the typical 'basmati' aroma. These observations on the quantity of volatiles from scented and non-scented varieties revealed that there are differences in this respect, among 'indica' and 'japonica' rices. Yazima et al (1979) reported an yield of only 0.9 ppm. from the scented japonica variety. The varieties studied in the present investigation gave an yield of 10.43 mg/kg to 33.80 mg/kg of cooked rice, suggesting that there are inherent differences with respect to the quantity of volatiles between indica and japonica rices.

The comparision of mean values of volatiles among scented rice varieties, with known gradation of scent such as high, medium and low scented varieties is shown in Table 9. The differences were statistically significant as evidenced by the 't' values. These results reveal that even among the scented varieties, there exists a clear difference in the quantity of volatiles and there is correlation between the degree of aroma and the quantity of volatiles. These observations suggest that, based on the quantitative studies of volatiles, we can distinguish different varieties with various degrees of aroma.

The analysis of volatiles from the variety Randhunipagalu' in a Gas Chromatograph equipped with a device to observe the smell of the separated fractions, showed that a small peak in the initial stages of the chromatogram might be responsible for the "Basmati flavour (Fig. 6). The separated fraction representing this peak gave a sharp odor and all the evaluators recorded that the flavour was that of the typical 'basmati' aroma. Other flavors observed were that of earthy, soily and naphthalene like. These observations show that the compound responsible for

the basmati flavour lies in the early stages of the chromatogram and its concentration is also very low. Tsuzuki et al (1975 a), found that most of the flavour components are observed within five minutes of the retention time and concluded that fragrant compounds melt out at a very low temperature.

The analytical GLC studies of volatiles from four scented and one non-scented varieties (Figs.7..11) exhibited the presence of 17 to 19 peaks. There were no qualitative differences among the varieties, and only the height of certain peaks such as 1, 2, and 6 varied between the varieties. Tsuzuki et al (1975 a) in their GLC studies of the flavour components of three scented and one ordinary variety, found that 19 peaks were present in every variety. After testing the flavour components at various time intervals, they concluded that as the cooking duration increases, each peak becomes prominent. It was observed that variation in the height of certain peaks exist and only quantitative differences were present between the varieties. Hayashi (1974) also showed that there were no qualitative differences between the scented and ordinary rice. Tsuzuki et al (1975 b) did not find any

differences in the carbonyl compounds between scented and ordinary rices, by TLC and GLC studies of 2, 4, DNP derivatives. After comparing the varieties by Co-chromatogram method, they observed that there were no qualitative differences between the varieties and concluded that scented varieties had higher carbonyl compounds than the ordinary variety. In the present study also, there were no qualitative differences and only certain quantitative differences were observed. In all the scented varieties, the separation was more clear and prominent in the initial stages of the chromatograms (upto 10 minutes) as compared to the non-scented variety where the peaks in this region were not well separated. In the scented varieties, peaks 1, 2 and 6 are more in height and quantitatively larger than in the ordinary variety. This pattern was more clear in the case of peak 6, which was the tallest peak in the scented varieties, while it was present only as a very small peak in the non-scented variety. These observation coupled with that from the GLC pattern with the aroma of the separated fractions (Fig. 6), suggest that the compound responsible for the basmati flavour is present in the initial

stages of the chromatogram and it could be peak 6. The presence of peak 6 in larger quantity in the scented varieties further testifier this. The major peaks such as 12, 13 ... 18, which are present in all the varieties as large peaks, could be the compounds responsible for the cooked rice flavour as this flavour is common to all the varieties.

V. Lipids and Fatty acid composition studies:

TLC studies of the extracted lipid samples from twenty scented and two non-scented varieties showed that there were no class differences in the varieties (Fig. 12). The intensities of the fraction 'free fatty acid' varied in some varieties and it was less intense in varieties like Basmati-370 and Jaya etc. Rao and Bandyopadhyay (1975) in their studies on the changes in lipids during banana ripening, showed that the TLC pattern of the lipids was identical in both the varieties during various stages of ripening. The observations on rice lipids also show that there are no qualitative differences among the different varieties.

The fatty acid composition was studied by GLC and this showed the presence of eight fatty acids in all the varieties, suggesting that there were no qualitative differences between the varieties (Table 10). Lee et al (1965) and Resurreccion and Juliano (1975), in their studies on the fatty acid composition of different rice varieties, found that the major components were palmitic, oleic and linoleic acids. Resurreccion and Juliano (1975) found lauric acid from milled rice which was absent in the present study. When the fatty acid compositions were compared to the values reported by these workers, there were slight discrepancies in them. Resurreccion and Juliano (1975) reported that linoleic acid was 46.1 and 45.2% from milled rice samples and oleic acid was about 22.6 to 23.1%. In the present study, the values ranged from 19.93 to 33.81% for linoleic acid and 37.30 to 49.26% for oleic acid, showing that the quantity of linoleic acid was less and the quantity of oleic acid was more in these varieties. These differences could be due to varietal, soil and climate differences. Yasumatsu and Moritaka (1964) showed that the soil, climate and storage temperature had an effect on the fatty acid composition, of two Japanese rice varieties.

There were quantitative differences in the composition of certain fatty acids among the varieties. In all the scented varieties, the quantity of oleic acid was higher when compared to non-scented varieties. It ranged from 44.17 to 49.26% in the scented varieties, while it was only 37.30 and 38.6% in the non-scented varieties. Similar varietal differences were found in the banana pulp lipid, where one variety had higher quantity of linoleic acid than other variety (Rao and Bandyopadhyay 1978). These observations suggest that the presence of oleic acid in higher concentration in the scented varieties may have a role in the production of volatile flavour compounds, since oleic acid is known to be one of the precursor for these compounds (Tsuzuki et al 1975 a). The presence of oleic acid in higher quantity may lead to the production of volatile flavour components also in higher concentration and thus resulting into one or some of the components responsible for 'basmati' aroma. Till this date, no qualitative differences have been found between the volatile flavour compounds of scented and non-scented rices, except that of 1-pyrrolidine (a degradation product of the amino acid proline) which is present in scented rice only (Yazima et al 1979).

These workers concluded that the differences in the aroma of scented and ordinary rices are due to the difference in the balance or proportion of the various volatile flavour components, and found that the scented variety had more quantity of indole than ordinary rice variety and the latter contained more amounts of n-hexanol and n-hexanal. Based on these observations, it can be assumed that the presence of oleic acid in higher concentration in scented rices as found in the present study, has a definite role in the production of volatile flavour components responsible for the basmati flavour.

VI. Amino acid composition studies:

Amino acid composition studies in twelve scented and one non-scented variety, showed a wide variation for most of the amino acids among the different varieties (Table 11). Glycine, aspartic and glutamic acids had the highest values of variation. Among the essential amino acids, lysine exhibited maximum variability, followed by valine, histidine, threonine and methionine. These observations are in contrast to those of Houston et al (1969) who obtained little

variation in the essential amino acid pattern of milled rice samples.

The quantity of proline was higher in all the scented varieties than in non-scented variety (Fig.14). These observations suggest that, proline might be acting as a precursor to some of the volatile flavour components such as 1-pyrrolidine, which is formed by the degradation of proline. This compound was shown to be present in the volatile flavour components of scented rice (Yazima et al 1979). Since aroma results from the interaction of different types of compounds present in different concentrations, higher concentration of proline might be producing more amounts of flavour components which have a role in the production of 'basmati' aroma. Alanine also was present in higher amounts (Fig. 14) in the scented varieties. Alanine is also one of the precursors for the flavour compounds such as Acetaldehyde which has been reported in rice, arising due to Strecker's degradation on heating with sugars (Table 13). The presence of alanine in higher concentration in the scented varieties suggests that during cooking this amino acid might be degraded, to produce the flavour components, which by

TABLE 13

Aldehydes in rice	Amino acids as precursor in the Strecker degradation
Acetaldehyde CH_3CHO	Alanine $CH_3-CH(NH_2)-COOH$ Cysteine $HS-CH_2-CH(NH_2)-COOH$ Cystine $S-CH_2-CH(NH_2)-COOH$ $S-CH_2-CH(NH_2)-COOH$
Propionaldehyde CH_3-CH_2-CHO	α-Amino butyric acid $CH_3-CH_2-CH(NH_2)-COOH$
n-Valeraldehyde $CH_3-CH_2-CH_2-CH_2-CHO$	Norleucine $CH_3-CH_2-CH_2-CH_2-CH(NH_2)-COOH$
n-Caproaldehyde $CH_3-CH_2-CH_2-CH_2-CH_2-CHO$	α-Amino heptylic acid $CH_3-CH_2-CH_2-CH_2-CH_2-CH(NH_2)-COOH$

interacting with other flavour compounds may have a role in the formation of 'basmati' aroma.

The essential amino acid composition of the varieties was compared to that of the FAO recommended values (Fig. 15). For limiting amino acids, like lysine, most of the scented varieties had comparable values with the recommended values and one variety Type-3 even exceeded this value. The other varieties including the non-scented one had lower values. For methionine, all the scented varieties had higher values than the non-scented one and were comparable to the FAO values, while the non-scented variety had lower value than this. For leucine content, almost all the varieties had higher value than FAO values, while for isoleucine content all the varieties had lower values than the recommended value. For threonine content, all the varieties with few exceptions had comparable value with that of FAO value and the varieties, Randhunipagalu, Type-3 and Bindli exhibited maximum values for this. For valine content, varieties Randhunipagalu, Type-3 and Basmati-370 had the highest values and for pheylalanine content, all the varieties exceeded the FAO standards and Type-3 had

highest value for it followed by Randhunipagalu, SO-19 etc.

These observation on the essential amino acid composition of scented varieties, clearly suggest that these varieties have superior nutritional qualities due to their high essential amino acid composition. In view of this, some of these varieties could be successfully utilised in the breeding of varieties combining basmati qualities coupled with better amino acid composition. Varieties like Type-3, Randhunipagalu and Basmati-370 which exhibited highest values for most of the essential amino acids in particular could be utilised for such programmes.

SUMMARY

The present study deals with various aspects of scented (basmati) rices, like genetics of 'aroma', biochemical studies involving esterase isoenzymes and lipoxygenase enzyme, volatile flavor components, lipids, fatty acid and amino acid composition, etc.

The genetics of 'aroma' was studied among the segregating material of five crosses involving scented and non-scented varieties and a cross involving scented varieties. In crosses with scented and non-scented, the F_1 plants were all non-scented and the F_2 plants segregated into scented and non-scented and the F_2 data fitted in the monogenic ratio of 3:1 (non-scented: scented), whereas the F_1 and F_2 plants in the cross involving scented, were scented. These observations suggest that 'aroma' in rice is governed by a single recessive gene.

Esterase isoenzyme pattern was studied in 5 day old seedlings of thirty scented and six non-scented rice varieties. Non-scented rices had two specific bands, band 8 (Rf 0.46) and band 12(Rf 0.63),

which were absent in all the scented rice varieties. Among the scented rice varieties, there were differences in the zymogram pattern and were grouped under four categories. These observations support that esterase isoenzyme pattern may be helpful in characterisation of scented and non-scented varieties.

Lipoxygenase enzyme was studied quantitatively in ten scented and two non-scented rice varieties and was tested at different pH levels viz., 5,6,7,8,9 and 10, to know the optimum pH for its activity. All the varieties except IR 8 exhibited the optimum activity at pH 8, whereas IR 8 had the maximum activity at pH 6, which could be due to the varietal differences. The enzyme activity was also studied at different time intervals and these kinetic studies exhibited similar patterns typical to lipoxidase. The scented rice varieties generally had lower values of specific activity ranging from 40-50 units/mg. protein and the non-scented varieties had the values of 80-85 units/mg. protein suggesting that scented rice varieties posses better storage qualities as this enzyme is mainly responsible for the production of off-flavors during storage.

The quantitative estimation of volatiles in eight scented and two non-scented varieties showed higher mean values of volatile flavor components in scented compared to non-scented varieties, and were statistically significant. The mean values between varieties with known gradation of scent, i.e., highly scented varieties vs. medium and low scented varieties and medium vs. low scented varieties were found to be statistically significant. These observations suggest that the scented rice varieties exhibit significantly higher amounts of volatiles and variation exists based on the nature of aroma.

The concentrates of volatiles from scented varieties were studied by GLC and the variety'Randhunipagalu' in a Gas chromatograph equipped with a device to smell the separated fractions, showed that the 'basmati flavor' was coming in the initial stages and that the quantity of the compound was very low. The concentrates of volatiles from four scented rice varieties and one non-scented variety were subjected to analytical GLC. The chromatograms showed total number of 17-19 peaks. Although there were no qualitative differences among the peaks, the peaks in the initial phase

of the chromatograms of the scented were more prominent and quantitatively larger. Peak No. 6 was predominant in all the scented rice varieties and was present only as a small peak in non-scented variety. These observations further suggest that the actual compound responsible for the 'basmati' flavor in the scented rice varieties is very low and peak No. 6, could be the compound responsible for the aroma in these varieties.

Lipids and fatty acid composition was studied in twenty scented and two non-scented rice varieties and these samples were subjected to TLC. The preleminary observations suggest that there were no major differences among the varieties studied.

Fatty acid composition studies revealed the presence of myristic, palmitoleic, stearic, palmitic, oleic, linoleic, linolenic and arachidic acids in all the varieties. There were no qualitative differences, but the scented rice varieties showed higher quantity of oleic acid compared to non-scented varieties which varied from 44.17% to 49.26% in the scented as compared to 37.3% and 38.6% in non-scented. As fatty acids are one of the precursors for volatile flavor

components, the presence of oleic acid in higher quantities may be due to its active involvement in biosynthesis of volatiles.

Amino acid composition studies with twelve scented and one non-scented varieties showed wide variation for most of the amino acids. Proline, and alanine which are known to be the precursors for certain volatile flavor components, were present in greater quantities in scented varieties than in non-scented ones. The essential amino acid pattern was compared among different varieties with that of FAO standards. Lysine, methionine, leucine, isoleucine, and threonine exhibited higher values in scented than the non-scented, and were comparable to that of FAO recommended values. Varieties like Type-3, far exceeded this value. These observations suggest that, most of the scented varieties have superior aminograms.

The present studies clearly suggests that due to simple monogenic inheritance of scentedness and with identifiable biochemical parameters, the varieties in desirable background can be developed and improved for greater yield for commercial utilisation as if to confirm the Darwin's statement 'Hard cash is a good criterion of genetic superiority' (Brewbaker 1964).

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