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STUDIES ON PROTEINS AND ISOZYMES IN AROMATIC AND NON AROMATIC RICE

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SUMMARY

SDS-PAGE of soluble proteins and storage proteins did not reveal any marked differences between the aromatic and non-aromatic varieties of rice. However, isozyme analysis of the two groups of rice varieties showed malate dehydrogenase to be monomorphic while eight other enzymes, *viz.* alcohol dehydrogenase, aspartate aminotransferase, esterase, glucose-6-phosphate isomerase, glutamate dehydrogenase, peroxidase, phosphoglucomutase and shikimate dehydrogenase to be polymorphic. Glucose-6-phosphate isomerase and peroxidase isozyme patterns were distinct and could be used for distinguishing non-basmati aromatic varieties while phosphoglucomutase isozyme pattern could be useful in identifying the basmati aromatic varieties. Dendrogram constructed, based on isozyme data revealed three major clusters of rice varieties. Isozyme polymorphism may be used for differentiation and identification of rice cultivars and for utilization in a rice varietal improvement programme.

Key words: **Aromatic, isozymes, non-aromatic, rice, storage proteins**

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for over 60% of the world population. A number of rice varieties are grown globally but the aromatic ones are highly valued in Asia and also have wider acceptance in Europe (Berner and Hoff 1986) and United States (Brook 1989). By virtue of aroma and special consumer preference, aromatic varieties command premium in the market as compared to non-aromatic varieties. In view of this, breeding efforts are directed to develop high yielding aromatic rice varieties to meet the export demand.

In self pollinating species, electrophoretic pattern of seed protein and isozyme has been utilized to differentiate between varieties, the criteria being the presence or absence of specific bands at a regular position on the polyacrylamide gel (*Arcioni et al.* 1980). Varietal differences in electrophoretic zymograms of rice seed proteins have been well documented (Sarkar and Bose 1984, Damardjati *et al.* 1985, Chen *et al.* 1987). Electrophoretically identifiable isozymes have been utilized for the classification of varieties within *O. sativa* (Glaszmann 1987). Isozymes are tissue, stage and species specific and often represent efficient genetic markers. Knowledge of the isozymic forms may allow a better understanding of the aromatic and non-aromatic character in the rice varieties. In the present study, therefore, SDS-PAGE of soluble and storage proteins and electrophoretic patterns of various isozymes in aromatic and non-aromatic rice varieties have been studied to see the difference, if any, between the groups and to develop biochemical markers for distinguishing aromatic from non-aromatic rice varieties.

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MATERIALS AND METHODS

Thirteen varieties of rice were taken in the present study (Table 1) and the aromatic varieties have been classfied into two groups based on the grain length and width. Soluble proteins were extracted by hand grinding the endosperm, soaked overnight in the extraction buffer (50 mM Tris-HCl, pH 7.6; 5 mM β-mercaptoethanol) in the ratio 1 : 4 (w/v). For isozyme study, seven-day-old etiolated seedlings were ground with 50 mM Tris-HCl buffer (pH 7.6) containing 5 mM each of β-mercaptoethanol and EDTA in the ratio 1:2 (w/v). For the extraction of peroxidase, the buffer without β-mercaptoethanol was used. The homogenate was centrifuged at 15,000 × g for 10 min. Supernatants obtained were used for studying soluble proteins and isozyme patterns respectively. Storage proteins were extracted as per the method described by Kumamaru *et al.* (1988). Extraction was done from single grain by hand grinding in 0.5 ml of 50 mM KH_2PO_4-NaOH, pH 6.8 containing 8M urea, 4% SDS, 20% glycerine and 5% β-mercaptoethanol. The homogenate was sonicated for two minutes and then centrifuged at 15,000 × g for 5 min. Soluble and storage proteins were electrophoresed on 12% and 14% SDS-polyacrylamide gels respectively accroding to the method of Laemmli (1970). Isozymes were separated on 7% polyacrylamide gel using an anionic system (Davis 1964) and stained as described by Vallejos (1983) for alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), esterase (EST), glucose-6-phosphate isomerase (GPI), glutamate dehydrogenase (GDH), malate dehydrogenase (MDH), peroxidase (POX), phosphoglucomutase (PGM) and shikimate dehydrogenase (SDH). The bands were scored for construction of dendrogram using Jaccard's index. Data were entered as presence/absence of bands ignoring the intensity.

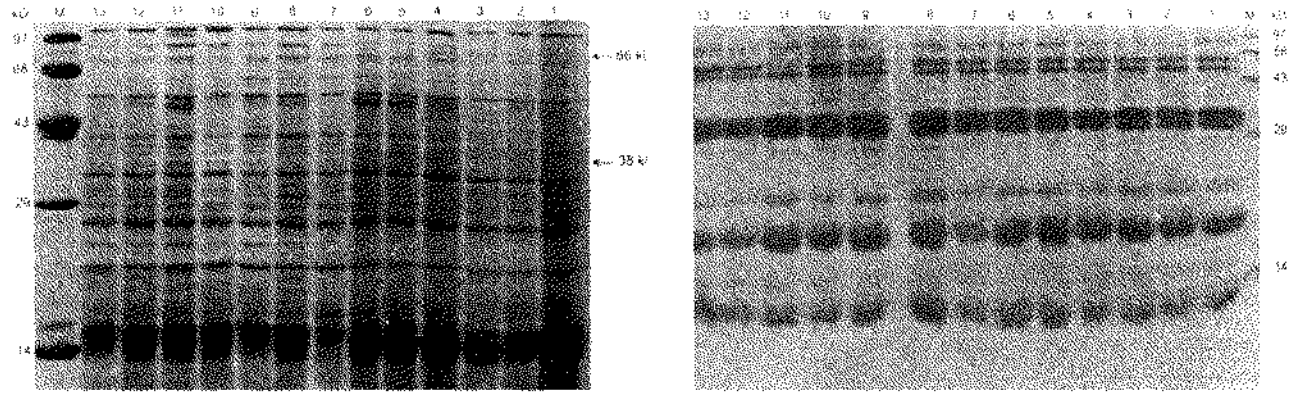
RESULTS AND DISCUSSION

Soluble protein pattern : SDS-PAGE of soluble proteins showed 31-39 polypetide bands in the aromatic and non-aromatic rice varieties (Plate 1a). Nine polypeptides with MWs 105, 53, 42, 33, 26, 22, 16, 15 and 14 kD were present in all the varieties. Seventeen minor bands with MW ranging from 28 to 83 kD were also observed. Steenson and Sathe (1995) found three major protein subunits with MWs 14.5, 22.4 and 33.1 kD which are in agreement with the present study. However, no distinct qualitative or quantitative differences were observed between aromatic and non-aromatic rice varieties. Electrophoretic variations were observed for some minor bands as two minor bands of MW 38 kD and 86 kD were observed only in the non-aromatic rice varieties. Another minor band of MW 47.5 kD was observed in basmati aromatic varieties.

Storage protein pattern : SDS-PAGE of storage proteins was almost similar among rice varieties (Plate 1b). All the rice varieties had sixteen protein subunits. An extra minor band of MW 69 kD was observed only in the non-aromatic varieties, which may possibly represent association with non-aromatic character of the varieties. Nine prominent

Table 1. List of varieties used in the present study.

No.	Variety	Type	Source
1	TTB-196-B-29-1-23-1	Non-basmati aromatic	Assam
2	TTB-196-B-43-2-4-1	Non-basmati aromatic	Assam
3	TTB-196-B-29-1-22-2	Non-basmati aromatic	Assam
4	TTB-196-B-43-2-9-1	Non-basmati aromatic	Assam
5	TTB-196-B-29-1-2-1	Non-basmati aromatic	Assam
6	TTB-196-B-29-1-15-5	Non-basmati aromatic	Assam
7	Basmati-370	Basmati aromatic	IARI, New Delhi
8	Pusa Basmati-1	Basmati aromatic	IARI, New Delhi
9	Karnal Local	Basmati aromatic	IARI, New Delhi
10	Jaya	Non-aromatic	IARI, New Delhi
11	Pusa-834	Non-aromatic	IARI, New Delhi
12	Lachit	Non-aromatic	Assam
13	Chilarai	Non-aromatic	Assam



1a 1b

Plate 1. SDS-PAGE pattern of soluble and storage proteins from rice varieties. (a) Soluble proteins, (b) Storage proteins. Lane 1. TTB-196-B-29-1-23-1, 2. TTB-196-B-43-2-4-1, 3. TTB-196-B-29-1-22-2, 4. TTB-196-B-43-2-9-1, 5. TTB-196-B-29-1-2-1, 6. TTB-196-B-29-1-15-5, 7. Basmati-370, 8. Pusa Basmati-1, 9. Karnal Local, 10. Jaya, 11. Pusa-834, 12. Lachit, 13. Chilarai, M : Protein molecular weight marker.

bands with MWs 65, 61, 32, 31, 28, 19, 14 and 11 kD were common in all the varieties. Kumamaru *et al.* (1988) also observed seven major bands with MWs 57, 37-39, 26, 22-23, 16, 13 and 10 kD on SDS-PAGE of storage proteins among rice varieties.

Qualitative protein markers for major proteins in rice are rare (Chen and Chen 1989). Various attempts have been made for characterization of rice proteins using native PAGE and SDS-PAGE, but the electrophoretic variations have been observed mostly in minor bands (Damardjati *et al.* 1985, Chen *et al.* 1987). Since variations were observed only in the minor bands in the present study also, it is difficult to conclude any relationship of aromatic and non-aromatic character on the basis of electrophoretic banding pattern of soluble and storage proteins.

Isoenzyme pattern : Polymorphism in the aromatic and non-aromatic rice varieties was detected in eight out of nine enzymes studied. MDH showed a monomorphic zymogram. The basmati aromatic var. Karnal Local, differed from rest of the varieties in having a higher mobility band for ADH (Plate 2a) as well as GDH) (Plate 2b). Two groups of AAT isozymes were observed. The varieties 1, 2, 3, 5 & 6 had lower mobility AAT band than the rest of the varieties (Plate 2c). Romero *et al.* (1993) too observed two forms of AAT in rice.

Both qualitative and quantitative differences in the isozyme pattern for EST (Plate 2d) and POX (Plate 2e) were observed in the present investigation. Fuentes *et al.* (1994) described similar polymorphism for esterase. Reddy and Reddy (1987) reported an esterase isozyme with Rm 0.9 specific to the non-aromatic rice. However, in the present study all the varieties, irrespective of aromatic or non-aromatic character, possessed this isozyme with Rm 0.9. No distinction could however be made between the aromatic and non-aromatic group. The non-basmati aromatic varieties showed a characteristic POX isozyme band at Rm 0.30 which was absent in the rest of the varieties. Glucose-6-phosphate isomerase (GPI) appeared to be polymorphic with three isozymes having a pattern similar to that observed by Romero *et al.* (1993). Although no clear distinction was observed between aromatic and non-aromatic groups with this enzyme, a characteristic band with Rm 0.31 was observed only in the non-basmati aromatic varieties (Plate 2f). Thus, these two enzyme patterns (POX and GPI) could be useful in distinguishing the non-basmati aromatic varieties from the rest.

PGM from the basmati aromatic varieties differed from the other varieties with a characteristic band pattern (Plate 2g). An intense band with Rm 0.38 was present in the non-basmati aromatic and non-aromatic rice varieties only. This enzyme could be used in distinguishing basmati aromatic varieties from the rest as three bands with Rm values 0.40, 0.42 and 0.44 were observed in Karnal Local, Basmati-370 and Pusa Basmati-1 respectively. In case of SDH, all the aromatic rice varieties possessed three

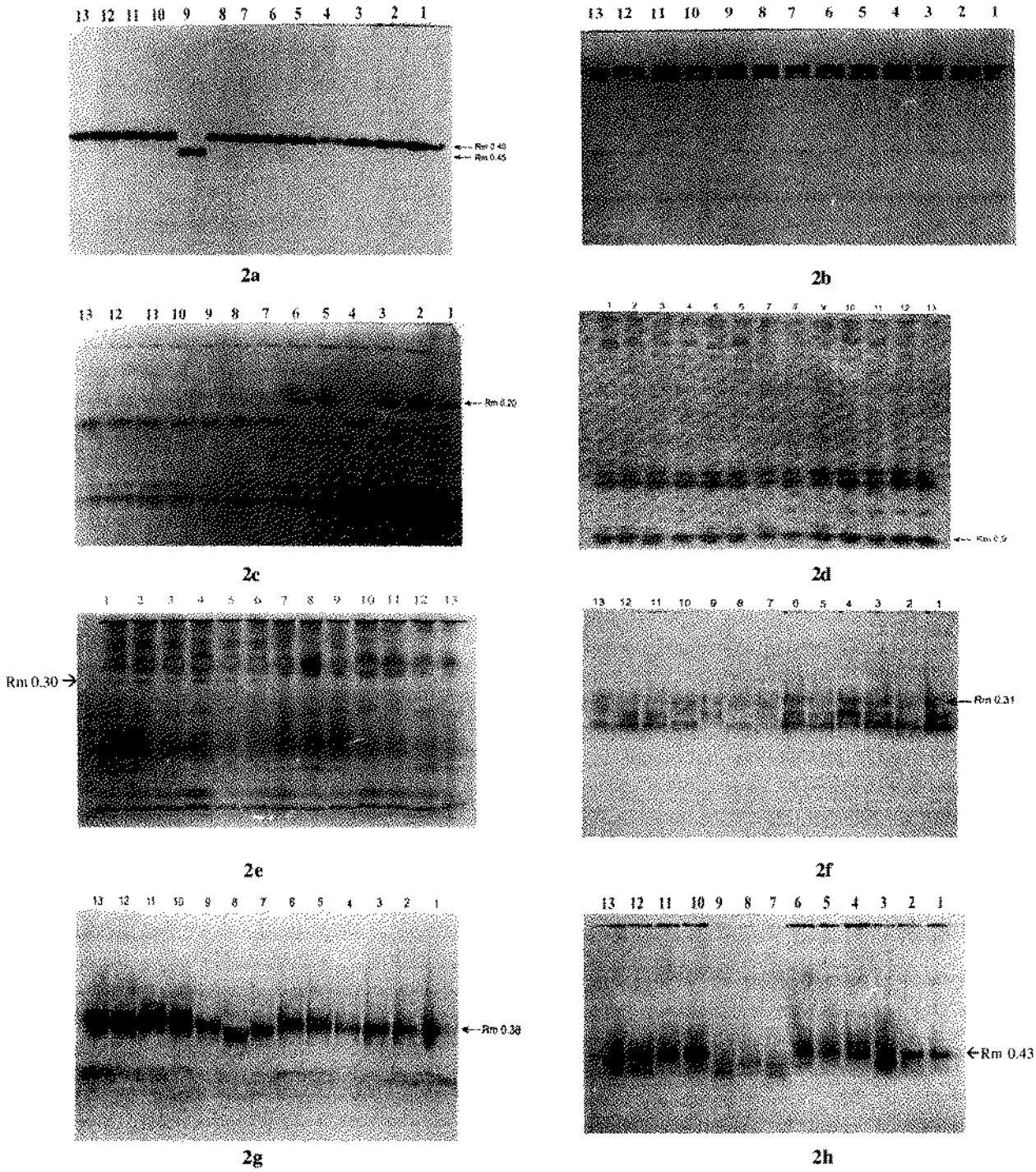


Plate 2. Isozyme pattern in the seven days old seedlings of the rice varieties.
(a) ADH (Alcohol dehydrogenase), (b) GDH (Glutamate dehydrogenase), (c) AAT (Aspartate aminotransferase), (d) EST (Esterase), (e) POX (Peroxidase), (f) GPI (Glucose-6-phosphate isomerase), (g) PGM (Phosphoglucomutase), (h) SDH (Shikimate dehydrogenase). Lane 1. TTB-196-B-29-1-23-1, 2. TTB-196-B-43-2-4-1, 3. TTB-196-B-29-1-22-2, 4. TTB-196-B-43-2-9-1, 5. TTB-196-B-29-1-2-1, 6. TTB-196-B-29-1-15-5, 7. Basmati-370, 8. Pusa Basmati-1, 9. Karnal Local, 10. Jaya, 11. Pusa-834, 12. Lachit, 13. Chilarai.

isozymic forms except for TTB-23-1 which along with the non-aromatic varieties showed two forms (Plate 2h). This is in contrast to single monomeric band observed by Glaszmann *et al.* (1988) for homozygotes. Romero *et al.* (1988) observed two bands for SDH and this was attributed to the homoallelic variants at the *Sdh*-1 locus, which was reported to be polymorphic (Nagamine *et al.* 1992). Similar polymorphism was observed in the present study. The band with Rm 0.43 was absent in basmati aromatic rice varieties only.

Dendrogram constructed based on isozyme data showed three major clusters (Fig. 1). The first cluster included all the non-basmati aromatic varieties with three sub-clusters. The second cluster consisted of the four non-aromatic varieties with two sub-clusters. Based on isozyme data, the two non-aromatic varieties, Jaya and Pusa-834 could not be distinguished from one another. This might be due to similar expression of the gene activity as well as the activity of specific enzyme. The third cluster comprised of the basmati aromatic varieties with two sub-clusters. While Basmati-370 and Pusa Basmati-1 were about 75% similar, there was only 66% similarity between Basmati-370 and Karnal Local, based on the isozyme profile. Least similarity (46.8%) was observed between var. TTB-9-1 and Karnal Local (Table 2).

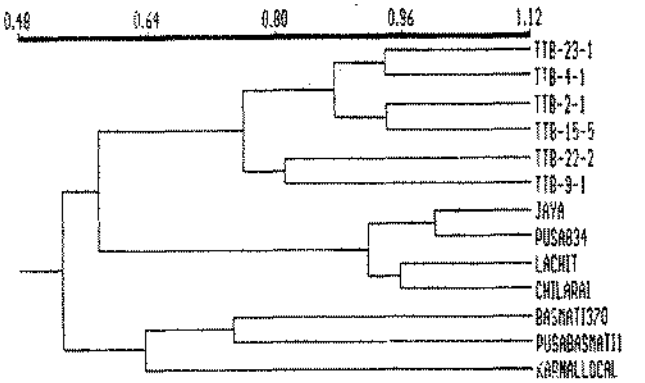


Fig. 1. Dendogram of rice varieties, constructed using UPGMA based on Jaccard's similarity coefficients.

No clear distinction could, however, be made between the aromatic and non-aromatic varieties of rice based on the isozyme patterns studied except for GPI and POX patterns which could be used for distinguishing the non-basmati aromatic varieties of rice. Characteristic banding pattern in PGM and absence of specific band in SDH could be of use for distinguishing basmati aromatic varieties from the rest. High mobility band for Karnal Local in case of ADH and GDH might be of specific use as the biochemical marker for the variety.

Table 2. Similarity matrix between each two varieties of aromatic and non-aromatic rice varieties (with respect to 9 isozymes).

	1	2	3	4	5	6	7	8	9	10	11	12	13
	TTB-23-1	TTB-4-1	TTB-22-2	TTB-9-1	TTB-2-1	TTB-15-5	Bas-370	PB-1	KL	Jaya	Pusa-834	Lachit	Chilarai
1	1.0000												
2	0.9388	1.0000											
3	0.7593	0.8113	1.0000										
4	0.6964	0.6842	0.8113	1.0000									
5	0.8627	0.8824	0.8462	0.7455	1.0000								
6	0.8824	0.8654	0.7963	0.7321	0.9400	1.0000							
7	0.5667	0.5833	0.6379	0.5833	0.5574	0.5738	1.0000						
8	0.4769	0.4923	0.5397	0.5645	0.4923	0.5077	0.7455	1.0000					
9	0.4762	0.4921	0.5161	0.4688	0.4921	0.5079	0.6607	0.6102	1.0000				
10	0.5667	0.5574	0.5833	0.6102	0.5833	0.6271	0.5161	0.5484	0.6034	1.0000			
11	0.5667	0.5574	0.5833	0.6102	0.5833	0.6271	0.5161	0.5484	0.6034	1.0000	1.0000		
12	0.5424	0.5333	0.5593	0.5862	0.5593	0.6034	0.4918	0.5000	0.5789	0.9362	0.9362	1.0000	
13	0.5424	0.5333	0.5862	0.6140	0.5862	0.5763	0.4918	0.5000	0.5789	0.8958	0.8958	0.9556	1.0000

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