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RICE VARIETAL IDENTIFICATION BY ELECTROPHORETIC VARIANTS OF SALT SOLUBLE SEED PROTEINS AND SEED MORPHOLOGY

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SUMMARY

Efficacy of certain physico-chemical characters of grain and electrophoretic method (SDS-PAGE) was tested in rice varietal identification. SDS-PAGE of ten rice varieties resulted in scoring of twenty polymorphic bands. Out of these distinct presence/absence was exhibited by band no. 2 (Rm 0.23), 3 (Rm 0.26), 5 (Rm0.30), 6 (Rm 0.32), 7 (Rm 0.34), 8 (Rm 0.35), 12 (Rm 0.44) and 23 (Rm 0.97). The varieties were distinguishable from each other by determining the presence and/or absence of specific protein bands and their intensity in electrophoregrams. Polypeptide polymorphism along with 'seed keys' may be used for differentiation and identification of rice cultivars and for utilization in a rice varietal improvement programme.

Key words: **Protein marker, rice, SDS-PAGE, seed keys, varietal identification.**

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop of the world. It is staple food for more than 60 per cent of world population. Seed protein profiles can be used for distinguishing varieties (Baishya *et al.* 2003, Lamani *et al.* 2003) and testing genetic purity (Varier *et al.*1997) of seed-lots since they directly reflect genomic composition of varieties (Goyal *et al.*2003). Protein bands detected from electrophoresis of seed proteins have emerged as a potential tool in studies on genetic diversity and have effectively been employed for characterization and identification of varieties in several crop plants such as soybean (Lamani *et al.*2003), cotton (Cherry *et al.* 1970, Kapse *et al.*1985), cluster-bean (Goyal *et al.* 2003), chickpea (Singh *et al.*1992) including rice (Siddiq *et al.* 1972, Sarkar and Bose 1984, Chauhan and Nanda 1984, Padmavathi *et al*. 1999, Baishya *et al.* 2003). A number of studies have revealed that crop varieties could be distinguished on the basis of morphological characteristics of seed and seedling (Moris and Payne 1977, Payne and Koszykowashki 1977, Sivasubramanian and Ramkrishnan 1978, Agarwal and Pawar 1990 and Lamani *et al.* 2003).

In rice also various physico-chemical characters of the grain namely seed size, kernel colour, seed shape, phenol reaction have been utilized for cultivar identification (Sivasubramanian and Ramkrishnan 1978, Chauhan and Nanda 1984). In the present investigation, an attempt has been made to distinguish the rice varieties on the basis of electrophoregrams of seed proteins using SDS-PAGE coupled with seed morphological characters.

MATERIALS AND METHODS

The varieties of rice from different groups such as early (Karjat-184, Ratna), midlate (Jaya, Karjat-3), late (Ratnagiri-2, Karjat-4), salt tolerant (Panvel-1, Panvel-2) and hybrids (Sahyadri, Sahyadri-2) were studied under

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this investigation. A representative sample of 10 decorticated rice grains was used to record length and breadth (Anonymous 2003). The phenol test and electrophoretic studies were conducted as suggested by Chauhan and Nanda (1984) and Sengupta and Chattopadhyay (2000), respectively with suitable following modifications.

Phenol test: Phenol test was done using various concentrations (0.5-2.0%) of phenol reagent in distilled water to find out optimum concentration. Representative samples of ten rice varieties were placed on filter paper (Whatman no. 42) in a Petridish at room temperature (30 ± 3^0 C), to which 5 ml solution was added. The observations were recorded after 24 hours.

Electrophoresis: Decoated 8-10 seeds from each variety were crushed to powder using mortar and pestle. Proteins were extracted by soaking finely ground seed in eppendorfs (1.5 ml) overnight in protein extraction solution (0.6 M Tris HCl pH 6.8; 4% SDS, 20% Glycerol and 5% β-mercaptoethanol). Thereafter, the proteins were denatured by boiling in water bath for 10 min. and then centrifuged at 12000 rpm for 10 minutes in refrigerated centrifuge. The clear supernatant was used for electrophoresis.

The denatured protein samples were electrophoresed in one dimensional, 1 mm thick 12.5 per cent sodium dodecyl sulfate polyacrylamide gel following Dadlani and Varier 1993. The SDS-PAGE was conducted by standard methods (Dadlani 1993, Cooke 1999) using 12.5 per cent acrylamide as separating slab gel (1.875 M Tris HCl pH 8.8) and 4 per cent acrylamide as stacking slab gel (0.6 M Tris HCl, pH 6.8). Each protein sample of 20 µls was loaded in wells of slab gel. Constant current of 35 mA was passed till tracking dye reached the base of the stacking gel, and then the current flow was increased to 40 mA until tracking dye (Bromophenol blue) reached the anode end. Gel staining was carried out in Coomassie Brilliant blue R (Solution 1%, 10ml) containing methanol, acetic acid (15%, 100ml). The gels were destained for 6 hrs by repeated washings with methanol : acetone : chloroform (1:1:2 v/v/v) until bands become prominent and resolutive.

The relative mobilities (Rm) value of each band was calculated and protein electrophoregrams were analysed for presence and/or absence of protein bands. The similarity index value for each pair of ten rice varieties was calculated from polypeptide banding pattern using the formula (Vaughan and Denford 1968).

$$S.I. = \frac{\text{No. of pairs of similar bands}}{\text{(No. of similar bands + no. of different bands)}} \times 100$$

RESULTS AND DISCUSSION

Varieties were classified on the basis of dimensions of decorticated grain length (mm), decorticated grain breadth (mm), decorticated grain shape, hypocotyls length of seedling (mm) (Maeda *et al*. 1995, El Aidy *et al*. 2000). Based on decorticated grain length and length-to breadth ratio all the varieties were grouped in two and four classes, respectively (Anonymous 2003). On the basis of decorticated grain length varieties Ratna, Panvel-2 and Sahyadri were classified as 'long' and remaining seven as 'medium'. According to Length: Breadth ratio, the varieties were grouped into 'short bold' (Jaya, Ratnagiri-2), 'medium slender' (Karjat-3, Karjat-4 and Panvel-1), 'long slender' (Karjat 184, Ratna, Sahyadri and Sahyadri-2) and 'extra long slender' (Panvel-2) (Table 1). Out of all varieties, hybrid Sahyadri-1 exhibited maximum 1000 seed weight (27.84 g) and late variety Karjat-4 (12.56 g) exhibited the lowest.

Phenol reaction, which is an index of polyphenol oxidase activity, has been reported to be associated with paddy discolouration during storage and could also be used in ascertaining the varietal purity (Abrol and Uprety 1972). Medium concentration (1%) of phenol solution was found to be quite effective in detecting the varietal difference (Table 1). Four types of phenol reactions were identified in the present investigation as 'no reaction' (Karjat-184), 'light brown' (Karjat-3, Ratnagiri-2), 'brown' (Ratna, Jaya, Sahyadri-1 and Sahyadri-2) and 'dark brown' (karjat-4, Panvel-1 and Panvel-2) (Table 1). This test further revealed differences in identical appearing varieties in other parameters such as seed shape. The varieties with identical decorticated grain length group as 'long' (Ratna, Panvel-2 and Sahyadri)

Table 1. Seed keys for identification of rice varieties.

Variety	1000 seed weight (g)	Grain shape*		Phenol reaction	Decorticated grain colour
		Decorticated grain length	L:B ratio		
Karjat-184	17.43	Medium	Long slender	No reaction	Light brown
Ratna	21.87	Long	Long slender	Brown	Light brown
Jaya	26.28	Medium	Short bold	Brown	Variegated brown
Karjat-3	21.60	Medium	Medium slender	Light brown	White
Ratnagiri-2	24.12	Medium	Short bold	Light brown	Light brown
Karjat-4	12.56	Medium	Medium slender	Dark brown	Light brown
Panvel-1	24.80	Medium	Medium slender	Dark brown	Variegated brown
Panvel-2	22.87	Long	Extra long slender	Dark brown	Variegated brown
Sahyadri	27.84	Long	Long slender	Brown	White
Sahyadri-2	23.96	Medium	Long slender	Brown	Variegated brown

(*Anonymous 2003)

revealed differences through phenol reaction as 'brown' (Ratna and Sahyadri) and 'dark brown' (Panvel-2). Similarly varieties with 'medium' decorticated grain length group (Karjat-184, Jaya, Karjat-3, Ratnagiri-2, Karjat-4, Panvel-1 and Sahyadri-2) revealed differences through phenol reaction as 'no reaction' (Karjat-184), 'brown' (Jaya and Sahyadri-2), 'light brown' (Karjat-3 and Ratngiri-2) and 'dark brown' (Karjat-4 and Panvel-1).

SDS-PAGE of seed proteins of 10 rice cultivars resulted in scoring of a maximum of twenty polypeptide bands (Plate 1 and Fig. 1). The results in general showed variation in the number and intensity of protein bands. The number of bands present in a variety ranged from 16 (Kajat-3) to 20 (Ratnagiri-2, Panvel-1 and Sahyadri). The protein band no. 2 (Rm 0.23), 3(Rm 0.26), 5 (Rm 0.30), 6 (Rm 0.32), 7 (Rm 0.34), 8 (Rm 0.35), 12 (Rm 0.44) and 23 (Rm 0.97) were most useful in

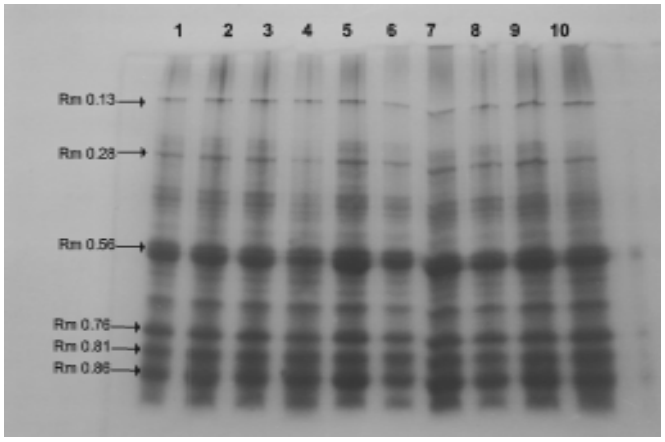

Plate 1. SDS-PAGE banding pattern of soluble seed proteins from rice varieties. Lane 1. Karjat-184, 2. Ratna, 3. Jaya, 4. Karjat-3, 5. Ratnagiri-2, 6. Karjat-4, 7. Panvel-1, 8. Panvel-2, 9. Sahyadri, 10. Sahyadri-2

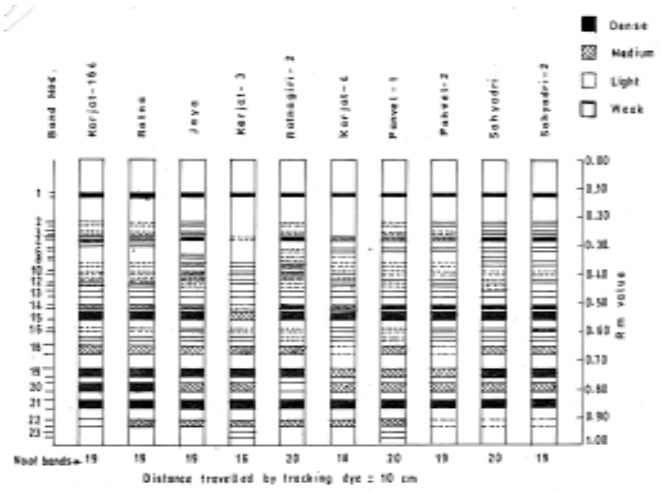

Fig. 1. Electrophoregrams of ten different varieties of Rice (*Oryza sativa* L.) [SDS-PAGE of soluble seed proteins]

distinguishing the varieties. The absence of these eight polypeptides may be attributed to either deletion or regulation of the structural gene(s) coding for these polypeptides (Brown *et al*. 1981).

The varieties were also found to differ in the intensity of corresponding bands. All varieties recorded common presence of band nos. 01 (Rm 0.13), 04 (Rm 0.28), 09 (Rm 0.37), 10 (Rm 0.40), 11 (Rm, 0.42), 13 (Rm 0.48), 14 (Rm 0.52), 15 (Rm 0.56), 16 (Rm 0.60), 17 (Rm 0.63), 18 (0.68), 19 (Rm 0.76), 20 (Rm 0.81), 21 (Rm 0.86) and 22 (Rm 0.93). These bands showed polymorphism in the intensity of bands as band no. 04 medium (Karjat 184, Ratna, Karjat-4, Panvel-1 and Panvel-2), dense (Jaya, Ratnagiri-2, Sahyadri and Sahyadri-2) and light (karjat-3); band no. 09 as weak, light and medium; band no. 10. as weak, light and medium; band no. 11 as weak and light; band no. 14 as light, medium and dense; band no. 15 as medium and dense; band no. 16 as weak, light and medium; band no. 17 as light and weak; band no. 16 as light and medium; band no. 19 as medium and dense; band no. 20 as medium and dense in all varieties. The band no. 01 (Rm 0.13), 13 (Rm 0.48) and band no. 21 (Rm 0.86) were monomorphically expressed in all ten varieties with dense, weak and dense intensities, respectively.

Table 2. SDS-PAGE profiles of seed proteins of rice varieties

Band	Rm value	Karjat-184	Ratna	Jaya	Karjat-3	Ratnagiri-2	Karjat-4	Panvel-1	Panvel-2	Sahyadri	Sahyadri-2
01	0.13	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
02	0.23	++	++	+	-	++	-	++	+	+	+
03	0.26	++	++	+	-	++	-	++	++	+	+
04	0.28	+++	+++	++++	++	++++	+++	+++	+++	++++	++++
05	0.30	+	+	-	-	-	-	-	-	-	-
06	0.32	-	-	-	-	++	+	+	+	+	+
07	0.34	-	-	+	-	-	+	-	-	+	-
08	0.35	-	-	-	-	++	-	-	-	-	-
09	0.37	++	++	+++	+	+++	++	++	++	+	+
10	0.40	++	++	+++	+	+++	++	++	++	++	++
11	0.42	++	+	++	+	++	++	+	+	++	+
12	0.44	+	+	++	-	++	+	+	+	++	+
13	0.48	+	+	+	+	+	+	+	+	+	+
14	0.52	+++	+++	+++	++	++++	+++	++++	++++	++++	++++
15	0.56	++++	++++	++++	+++	++++	++++	++++	++++	++++	++++
16	0.60	++	++	+	++	++	+	++	+	+	+++
17	0.63	+	+	+	+	++	+	++	+	+	+
18	0.68	+++	+++	+++	+++	+++	++	+++	++	+++	+++
19	0.76	++++	++++	++++	++++	++++	+++	+++	+++	++++	++++
20	0.81	++++	++++	+++	+++	++++	+++	+++	+++	+++	+++
21	0.86	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
22	0.93	+	+++	+++	+++	+++	+++	+++	++	++	++
23	0.97	-	-	+	+	-	-	+	-	-	-

Band intensity = ++++ Dense, +++ Medium, ++ Light, + Weak, - Absent

Table 3. Similarity matrix between each two varieties of rice

Variety	Ratna	Jaya	Karjat-3	Ratnagiri-2	Karjat-4	Panvel-1	Panvel-2	Sahyadri	Sahyadri-2
Karjat-184	89.47	47.36	40.00	46.15	59.45	61.53	47.36	46.15	47.36
Ratna		47.36	51.42	56.41	59.45	71.79	57.89	41.02	47.36
Jaya			40.00	61.53	54.05	30.76	36.84	76.92	57.89
Karjat-3				38.88	35.29	44.44	34.28	45.71	51.42
Ratnagiri-2					26.31	55.00	30.76	50.00	41.02
Karjat-4						63.15	70.27	57.89	37.83
Panvel-1							66.66	40.00	56.41
Panvel-2								51.28	66.66
Sahyadri									82.05

The distinct absence of band no. 02 and 03 (Karjat-3, Karjat-4); band no. 6 (Karjat 184, Ratna, Jaya, karjat-3); band no. 12 (Karjat-3) was observed. Similarly, distinct presence of band no. 05 (Karjat-184, Ratna); band no. 07 (Jaya, Karjat-3, Sahyadri-1); band no. 08 (Ratnagiri-2) and band no. 23 (Jaya, Karjat-3 and Panvel-1) was most useful in characterization of varieties. A percentage similarity between all possible pairs of varieties, based on protein bands is summarized in Table 3.

The present study revealed that no single test can be used for 'spotting' a particular variety. The ten rice varieties could be distinguished from each other on the basis of morphological characteristics of seeds (1000 seed weight, decorticated grain length) and biochemical characteristics like phenol reaction and by SDS-PAGE. A battery of tests may be useful in the identification of rice varieties, which could not be explained by seed keys and seedling morphology. Based on the findings of present investigation the possible identification schemes have been developed as seed keys and electrophoretic variants of salt soluble seed proteins.

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