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INTERSPECIFIC VARIATION OF TOTAL SEED PROTEIN IN WILD RICE GERMPLASM USING SDS-PAGE

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Abstract

Variation in seed protein of 14 wild rice species (*Oryza* spp.) along with cultivated rice species (*O. sativa*) was studied using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) to assess genetic diversity in the rice germplasm. SDS bands were scored as present (1) or absent (0) for protein sample of each genotype. On the basis of cluster analysis, four clusters were identified at a similarity level of 0.85. *O. nivara*, *O. rufipogon* and *O. sativa* with AA genomes constituted the first cluster. The second cluster comprised *O. punctata* of BB genome and wild rice species of CC genome i.e., *O. rhizomatis* and *O. officinalis*. However, it also contained *O. barthii* and *O. glumaepatula* of AA genome. *O. australiensis* with EE genome, and *O. latifolia*, *O. alta* and *O. grandiglumis* having CCDD genomes comprised the third cluster. The fourth cluster consisted of wild rice species, *O. brachyantha* with EE genome along with two other wild rice species, *O. longistaminata* and *O. meridionalis* of AA genome. Overall, on the basis of total seed protein, the grouping pattern of rice genotypes was mostly compatible with their genome status. The results of the present work depicted considerable interspecific genetic variation in the investigated germplasm for total seed protein. Moreover, the results obtained in this study also suggest that analysis of seed protein can also provide a better understanding of genetic affinity of the germplasm.

Introduction

Measurement of morphological traits alone may not serve a useful criterion for assessing genetic diversity of plant germplasm. The environmental influence on these traits may sometimes renders this measure relatively insensitive particularly where differences are very small. However, several molecular and biochemical analyses make it possible to establish differences at various taxonomic levels which in turns helps the researchers to assess genetic diversity in the investigated germplasm (Vaughan, 1983; Rabbani *et al.,* 2010; Pervaiz *et al.,* 2010; Mumtaz *et al.,* 2010). One of the biochemical methods extensively used in taxonomic and assessment of genetic diversity studies is the electrophoretic analysis of the seed proteins using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). These proteins are physiologically stable and easy to handle (Ladizinsky & Hymowitz, 1979). They operate at the level of gene product where the environment has very little influence (Feldman & Sears, 1981). Many rice genotypes have been characterized on the basis of electrophoresis profiles by SDS-PAGE (Montalavan *et al.,* 1995; Santhy *et al.,* 1998; Habib *et al.,* 2000; Sengupta & Chattopadhayy, 2000; Asghar *et al.,* 2004; Vivekananthan *et al.,* 2005). SDS-PAGE analysis has also been helpful in other crops to characterize and identify the germplasm. Ghafoor and Arshad (2008) characterized seventy seven pea genotypes for genetic divergence on the basis of seed protein profile using SDS-PAGE. Yousaf *et al.,* (2008) estimated intra and inter specific relationship of forty two accessions belonging to seven species of 4 different genera from the family *Solanaceae* through SDS-PAGE.

The objective of the present study was to assess genetic diversity in 14 wild rice species (*Oryza* spp.) obtained from International Rice Genebank Collection (IRGC), International Rice Research Institute (IRRI), Philippines along with cultivated rice species (*O. sativa*) on basis of total seed protein using SDS-PAGE.

Materials and Methods

The experiment was performed in the Evaluation Laboratory of Institute of Agriculture Biotechnology and Genetic Resources, National Agricultural Research Center (NARC), Islamabad, Pakistan. Germplasm of 14 wild species of rice provided by International Rice Genebank Collection (IRGC), International Rice Research Institute (IRRI), Philippines along with one commercial rice cultivar, Kinmaze was used. The list of wild rice species along with their IRGC accession number and source is given in Table 1. Rice cultivar, Kinmaze was used both as a representative of cultivated rice species, *Oryza sativa* and as a marker. In rice, the banding pattern of Kinmzae for total seed protein through SDS-PAGE is the most studied one (Aung *et al.,* 2001; Tian *et al.,* 2001; Qu *et al.,* 2003) and its banding pattern for most of the conserved rice seed proteins (57 kDa precursor bands of rice glutelin; 40 kDa acidic (α) glutelin band; 20 kDa basic (β) glutelin and 13 kDa prolamin bands) is usually considered as marker/standard for comparison purpose.

Preparation of seed sample: Seeds of each rice genotype were crushed and ground to fine powder with mortar and pestle and then 10 mg (0.01g) of seed flour of each rice genotype was weighed and put into 1.5 ml micro tube. To extract protein from flour, 200 µL of the protein extraction buffer (0.05 M Tris- HCl, 0.2 % SDS, 5 M Urea and 1% merceptethanol) was added into the tube and mixed well by vortex. Microtubes were then centrifuged at 15000 rpm for 10 min. at room temperature. The extracted proteins were recovered as clear supernatant and stored at -20 °C.

Table 1. International Rice Genebank Collection (IRGC) accession number and source countries of wild rice species (*Oryza* sp.) used in the study.

Wild rice species	IRGC accession	Source country
O. alta	100161	Brazil
O. australiensis	103303	Australia
O. barthii	100921	India
O. brachyantha	101233	Sierra Leone
O. grandiglumis	105144	Brazil
O. glumaepatula	100184	Cuba
O. latifolia	100966	Panama
O. longistaminata	101200	Nigeria
O. meridionalis	101145	Australia
O. nivara	104644	Thailand
O. officinalis	100954	India
O. punctata	100892	India
O. rhizomatis	103410	Sri Lanka
O. rufipogon	103308	Taiwan

Preparation of gel: Seed protein was analyzed through slab type SDS-PAGE followed by Laemmli (1970) using 15% polyacrylamide gel. In order to check reproducibility of the method separate gels were run three times under similar electrophoretic conditions. After electrophoresis, gels were put into a box containing staining solution and the box was shaken gently on electrical shaker for about 1 hour. Then staining solution was exchanged by destaining solution and shaken gently until disappearance of background of the gel. The gels were then analyzed and photographed.

Data analysis: SDS bands were scored as present (1) or absent (0) for protein sample of each genotype. The 1/0 matrix was used to calculate pair wise Dice similarity coefficients and the resulting matrix was used to construct an unweighted pair-group method with arithmetic means (UPGMA) phenogram using the SAHN (sequential, agglomerative, hierarchical, and nested) option of software package NTSYS-pc (Rohlf, 2000).

Results and Discussion

Fourteen wild species of rice (*Oryza* spp.) along with cultivated rice species (*O. sativa*) were analyzed using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS gels (Fig. 1) were carefully analyzed. During analysis a total of 34 bands were observed (Table 2). Both minor and major bands were kept under consideration. 57 kDa precursor bands of rice glutelin were marked as band numbers 10 and 11. Whereas, 40 kDa acidic (α) glutelin which is further fractioned into α_1, α_2 and α_3 subunits (Sarkar *et al.,* 1986; Kagawa *et al.,* 1988) were given band numbers 18, 19 and 20, respectively. β_1, β_2 and β_3 subunits of 20 kDa basic (β) glutelin (Sarkar *et al.,* 1986; Kagawa *et al.,* 1988) appeared as bands numbers 25, 26 and 27, respectively. While, 13 kDa prolamin bands were given band numbers 31, 32 and 33 (Fig. 2).

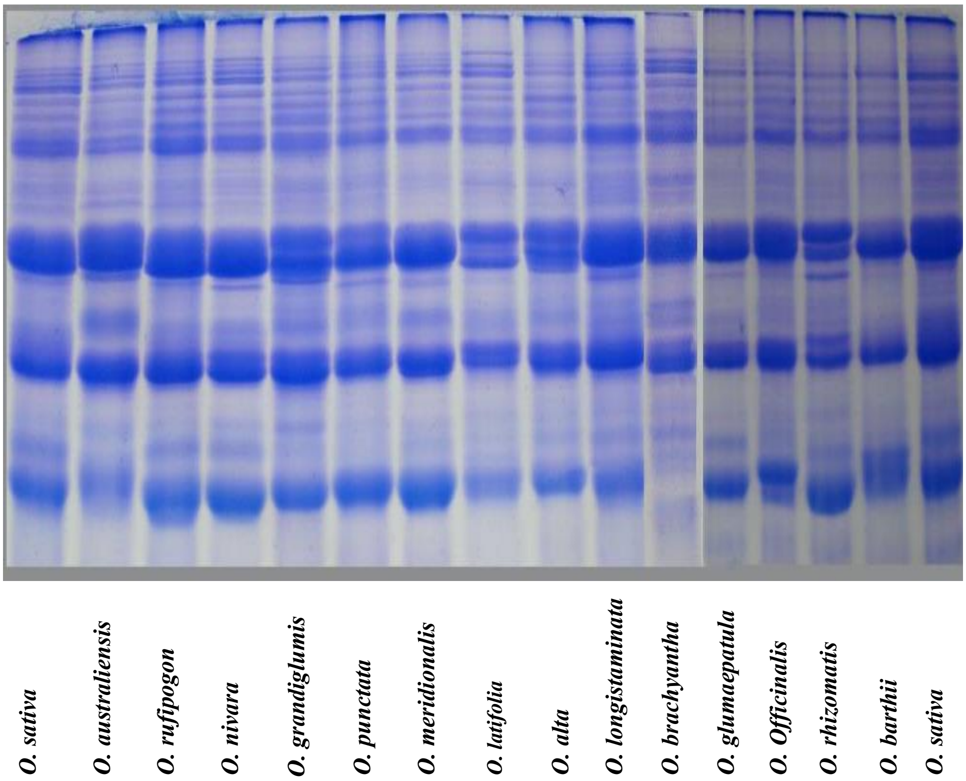

Fig. 1. SDS-PAGE analysis of total seed protein of 15 rice species.

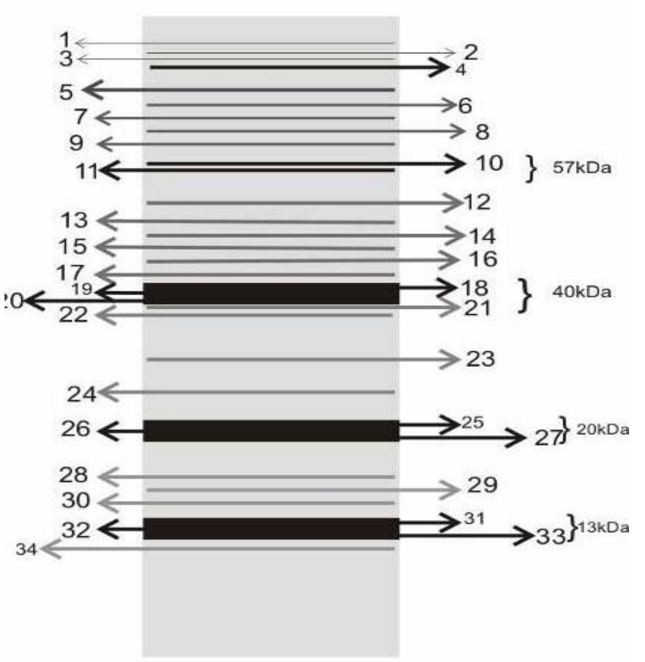

Fig. 2. A representative zymogram of SDS-PAGE of total seed protein of rice species to display positions of bands.

Dice similarity coefficient varied among the rice genotypes between 0.71 and 0.94 for total seed protein. *O. australiensis* and *O. brachyantha* showed minimum similarity coefficients with *O. glumaepatula* and *O. barthii*, respectively. Whereas, similarity coefficient of *O. alta* with *O. latifolia* was observed maximum. *O. sativa*, the cultivated rice species displayed minimum and maximum similarity coefficients with *O. australiensis* and *O. nivara*, respectively (Table 3). On the basis of cluster analysis, four clusters can be identified at a similarity level of 0.85 (Fig. 3). The first cluster contained wild rice species *O. nivara*, *O. rufipogon* and cultivated rice species, *O. sativa* of AA genome. The second cluster comprised *O. punctata* of BB genome and wild rice species *O. rhizomatis* and *O. officinalis* of CC genome. However, it also contained wild rice species *O. barthii* and *O. glumaepatula* of AA genome. *O. australiensis* with EE genome and *O. latifolia*, *O. alta* and *O. grandiglumis* with CCDD genome comprised the third cluster. The fourth cluster constituted wild rice species, *O. brachyantha* with EE genome along with two other wild rice species, *O. longistaminata* and *O. meridionalis* of AA genome. Moreover, band numbers 1-5, 9-11, 14, 15, 19, 20, 24 and 26 were present in all of the rice genotypes used in the study.

Table 2. Banding pattern of 15 rice species for total seed protein through SDS- PAGE.

	O. sat	***O. aus***	***O. ruf***	***O. niv***	***O. gra***	***O. pun***	***O. mer***	***O. lat***	***O. alt***	***O. lon***	***O. bra***	***O. bar***	***O. rhi***	***O. off***	***O. glu***
1.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6.	0	1	0	0	0	0	1	1	1	1	1	0	0	0	0
7.	1	0	1	1	1	1	0	0	0	0	0	1	1	1	1
8.	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0
9.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12.	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0
13.	1	0	1	1	0	1	0	0	0	0	1	1	1	1	0
14.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16.	0	1	1	1	1	1	0	1	1	1	1	0	0	0	0
17.	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1
18.	1	1	1	0	1	1	0	1	1	1	0	1	1	1	1
19.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
20.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
21.	1	1	1	0	1	0	0	0	1	0	0	1	0	1	0
22.	0	0	0	1	0	1	1	1	0	1	0	1	1	1	1
23.	0	1	0	0	1	1	0	0	0	1	1	0	1	1	0
24.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
25.	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
26.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27.	1	1	1	1	1	0	1	0	1	1	1	0	0	0	0
28.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
29.	1	0	1	1	1	0	1	1	1	1	1	0	0	0	1
30.	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0
31.	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0
32.	1	1	0	0	1	1	1	1	1	1	0	1	1	0	1
33.	1	0	1	1	0	0	1	0	0	1	0	0	1	1	1
34.	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0

1 = Band present; 0= Band absent

[a]*O. sat*: *O. sativa*; *O. aus*: *O. australiensis*; *O. ruf*: *O. rufipogon*; *O. niv*: *O. nivara*; *O. gra*: *O. grandiglumis*; *O. pun*: *O. punctata*; *O. mer*: *O. meridionalis*; *O. lat*: *O. latifolia*; *O. alt*: *O. alta*; *O. lon*: *O. longistaminata*; *O. brac*: *O. brachyantha*; *O. bar*: *O. barthii*; *O. rhi*: *O. rhizomatis*; *O. off*: *O. officinalis*; *O. glu*: *O. glumaepatula.*

Table 3. Dice similarity coefficients among 15 species^a of rice for total seed protein analyzed through SDS-PAGE.

Species	O.aus	O.ruf	O.niv	O.gra	O.pun	O.mer	O.lat	O.alt	O.lon	O.brac	O.bar	O.rhi	O.offi	O.glu
O. sat	0.77	0.88	0.90	0.89	0.82	0.82	0.78	0.85	0.84	0.80	0.84	0.82	0.86	0.83
O. aus		0.81	0.74	0.89	0.82	0.77	0.86	0.92	0.84	0.80	0.80	0.78	0.78	0.71
O. ruf			0.90	0.85	0.82	0.82	0.78	0.85	0.84	0.84	0.80	0.78	0.86	0.79
O. niv				0.83	0.88	0.83	0.84	0.82	0.85	0.86	0.82	0.84	0.88	0.85
O. gra					0.87	0.74	0.87	0.92	0.88	0.81	0.81	0.79	0.83	0.80
O. pun						0.75	0.88	0.82	0.85	0.82	0.90	0.92	0.92	0.85
O. mer							0.83	0.82	0.88	0.85	0.72	0.79	0.75	0.84
O. lat								0.94	0.89	0.82	0.82	0.80	0.80	0.85
O. alt									0.87	0.84	0.80	0.74	0.78	0.79
O. lon										0.87	0.75	0.81	0.81	0.82
O. brac											0.71	0.77	0.77	0.74
O. bar												0.90	0.90	0.83
O. rhi													0.92	0.89
O. offi														0.85

^{a}*O.sat*: *O. sativa*; *O.aus*: *O. australiensis*; *O.ruf*: *O. rufipogon*; *O.niv*: *O. nivara*; *O.gra*: *O. grandiglumis*; *O.pun*: *O. punctata*; *O.mer*: *O. merildonalis*; *O.lat*: *O. latifolia*; *O.alt*: *O. alta*; *O.lon*: *O. longistaminata*; *O.brac* : *O. brachyantha*; *O.bar*: *O. barthii*; *O.rhi*: *O. rhizomatis*; *O.off*: *O. officinalis*; *O.glu*: *O. glumaepatula*

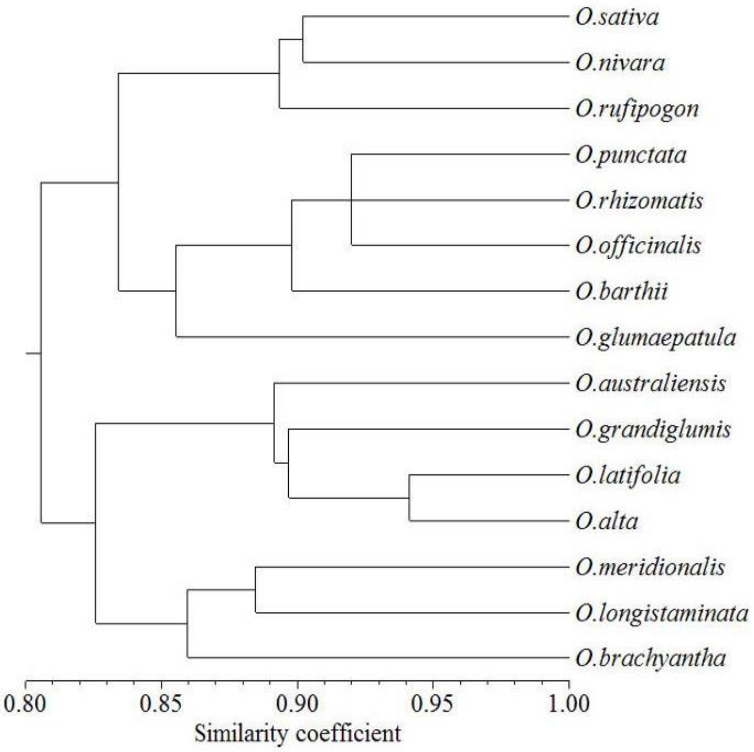

Fig. 3. Dendrogram of 15 rice species based on Dice similarity matrix for total seed protein analyzed through SDS-PAGE.

Genotypes in first cluster contained band numbers, 1-5, 7, 9-11, 13-15, 19, 20, 24-29, 31 and 33. Band numbers 6, 8 and 30 were absent in all the three genotypes of this cluster. Wild rice species, *O. rufipogon* of this cluster showed band number 12, whereas the same band was absent in other two genotypes of this cluster. Band number 16 was present in both wild rice species, *O. nivara* and *O. rufipogon* whereas it was absent in cultivated rice, *O. sativa*. Band number 17 was present in *O. sativa* and *O. nivara* but absent in *O. rufipogon*, whereas band number 18 was present in *O. sativa* and *O. rufipogon* but showed its absence in *O. nivara*. Both *O. sativa* and *O. rufipogon* displayed band number 21 while the same was absent in *O. nivara*. However, band number 22 was present only in *O. nivara* and absent in *O. sativa* and *O. rufipogon*. Band number 32 and 34 were present in cultivated rice, *O. sativa* but absent in wild rice species, *O. nivara* and *O. rufipogon*. It is also worth mentioning that band numbers 12, 16, 22 and 23 were absent in cultivated rice but these bands were present in either or both of wild rice species in this cluster.

Genotypes of the second cluster showed band numbers 1-5, 7, 9-11, 14, 15, 18-20, 22, 24 and 26. Band numbers 6, 8, 12, 27 and 34 appeared absent in all the genotypes of this cluster. Only one wild rice species, *O. glumaepatula* in this cluster didn't display band number 13, while the remaining genotypes of the cluster showed this band. Band number 16 was present only in *O. punctata* and was absent in other members of the cluster. *O. barthii* and *O. officinalis* manifested band number 21 whereas *O. glumaepatula*, *O. punctata* and *O. rhizomatis* didn't show this band. Band number 23 was present in *O. punctata*, *O. officinalis* and *O. rhizomatis*. Wild rice species *O. barthii* and *O. glumaepatula* with AA genomes, however, lacked this band. Band numbers 25, 28, 32 were present in all the wild rice species of this cluster except *O. barthii*, *O. glumaepatula* and *O. officinalis*, respectively. Only *O. glumaepatula* displayed band number 29, while the other genotypes of this cluster didn't manifest this band. *O. barthii* and *O. rhizomatis* gave band number 30, while the remaining genotypes lacked this band. Band number 31 was present in *O. barthii*, *O. punctata* and *O. officinalis*. Wild rice species *O. glumaepatula* and *O. rhizomatis*, however, didn't display this band. *O. glumaepatula*, *O. officinalis* and *O. rhizomatis* revealed band number 33 whereas *O. punctata* and *O. barthii* lacked this band.

Genotypes in the third cluster manifested band numbers 1-5, 8-11, 14-16, 18-20, 24-26, 28, 31 and 32. All of these genotypes, however, didn't display bands 12, 13 and 33. *O. grandiglumis* didn't show band number 6, whereas it was present in other genotypes of the cluster i.e. *O. australiensis*, *O. alta* and *O. latifolia*. The same genotype, however, showed band numbers 7 and 34, which were not displayed by other genotypes of the cluster. *O. alta*, *O. grandiglumis* and *O. latifolia* revealed band number 17, whereas *O. australiensis* lacked this band. Band number 21 was present in all the genotypes of this cluster except *O. latifolia*. However, *O. latifolia* showed band number 22, which was not revealed by any other genotype of the cluster. *O. australiensis* and *O. grandiglumis* displayed band number 23 whereas *O. alta* and *O. latifolia* lacked this band. *O. latifolia* and *O. australiensis* lacked band numbers 27 and 29, respectively. These two bands were, however, present in other genotypes of the cluster.

Genotypes of the fourth cluster displayed band numbers 1-6, 9-12, 14, 15, 19, 20 and 24-29. These genotypes, however, lacked band numbers 7, 8, 21 and 30. Band number 13 was absent in both *O. longistaminata* and *O. meridionalis* whereas *O. brachyantha* showed the same band. Band numbers 16 and 17 were not present in *O. meridionalis* whereas these bands were present in both *O. brachyantha* and *O. longistaminata*. Only *O. longistaminata* displayed band number 18, whereas this band was not present in other two wild rice species of this cluster i.e. *O. brachyantha* and *O. meridionalis*. Band number 22 was absent in *O. meridionalis* but present in both *O. brachyantha* and *O. longistaminta*. *O. longistaminata* displayed band numbers 31-34, whereas these bands were absent in *O. brachyantha*. *O. meridionalis*, however, displayed band numbers 32 and 33; while band numbers 31 and 34 appeared absent in this wild rice species.

Overall, on basis of total seed protein, the grouping pattern of rice genotypes was mostly compatible with their genome status as observed in seed protein analysis of cultivated and wild rice species through SDS-PAGE (Sarkar & Raina, 1992), in phylogenetic studies of rice species by AFLP markers (Aggarwal *et al.,* 1999) and in phylogenetic analysis of Adh1 and Adh2 genes of rice species (Ge *et al.,* 1999). However, the second cluster had *O. punctata* of BB genome, wild rice species *O. rhizomatis* and *O. officinalis* of CC genome, and wild rice species *O. barthii* and *O. glumaepatula* of AA genome. Earlier, studies of variations in mitochondrial, chloroplast and nuclear DNA have suggested multiple origins of *O. glumaepatula* and various accessions of *O. glumaepatula* have also shown similarity to *O. barthii* (Doi *et al.,* 2000) as observed in the present study. *O. brachyantha* with EE genome together with *O. longistaminata* and *O. meridionalis* of AA genome comprised the fourth cluster. In molecular studies of transposable element group *Tourist* in *Oryza*, *O. longistaminata* and *O. brachyantha* have shown resemblance with each other as observed in the present study on the basis of seed protein analysis (Zhang & Kochert, 1998). However, many taxonomic and molecular studies suggest otherwise (Vaughan & Morishma, 2003; Joshi *et al.,* 2000; Aggarwal *et al.,* 1999). *O. brachyantha* is a distinct *Oryza* species within its own section that appears to be more closely related to the genus *Leersia*.

The results of the present study depicted ample inter-specific genetic variation in the investigated germplasm for total seed protein which are compatible with the findings of many researchers. Zhan and Lin (1991) have also observed variation in protein of five classes of rice cultivars and six wild rice species through SDS-PAGE. Montalavan *et al.*, (1995) obtained similar results in a study of geographical distribution of different Brazilian rice varieties using seed protein polymorphism. They also concluded that electrophoretic analysis of seed protein could be used to estimate genetic relationship among different accessions. Similarly, Santhy *et al.,* (1998) and Habib *et al.,* (2000) characterized rice germplasm on the seed protein basis using SDS-PAGE. Asghar *et al.,* (2004) in a study of 20 accessions of rice also observed inter-specific variation on basis of seed protein analysis through SDS-PAGE.

Characterization of genetic diversity in wild rice species, in the present study, offers an opportunity for breeders for its exploitation in wide hybridization programs. Furthermore, the results obtained in this work also showed that SDS-PAGE analysis can provide an easy, low cost and quick way for the identification of wild rice accessions and also have a better knowledge of the genetic affinity of germplasm.

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