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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (2): 139-143, 2004
ISSN 1028-8880

Inter and Intra-Specific Variation in SDS-PAGE of Total Seed Protein in Rice (*Oryza sativa* L.) Germplasm

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Abstract: Plant gene pools are reservoirs of variations, which provide the raw material for crop improvement. Samples in the form of seeds representing the spectrum of genetic variation within cultivated species and their wild relatives. Each variety or a group of varieties exhibits characteristic banding pattern. On the basis of these patterns they can be identified accordingly. Electrophoretic patterns of the protein fractions are directly related to the genetic background of the proteins and can be used to certify the genetic makeup. SDS-PAGE is increasingly used to describe the genetic structure of crop germplasm identification. A total of twenty accessions of rice (*Oryza sativa* L.) germplasm were analyzed for total seed protein through SDS-PAGE, to ascertain the extent of genetic variation and its geographical distribution. A considerable variation in protein banding pattern was observed which was distributed to various geographical regions. Inter-specific variation was more as compared to intra-specific variation.

Key words: Germplasm, rice, *Oryza sativa*, SDS-PAGE, protein, seed

INTRODUCTION

Germplasm is a vital source in generating new plant types having desirable traits. It helps in increasing crop quality and production as well, that improve the level of human nutrition. Landraces are a useful source of genetic variation and the greater the variation in genes will result in large number of combinations of interest to plant breeders. Polygenic morphological traits also serve as genetic markers for various plant germplasm management and taxonomy. The measurement of morphological characters alone to access genetic diversity may not be very effective. The environmental effect on these characters renders this measure relatively insensitive, particularly where differences are very small. More sensitive markers are thus required. Protein markers are widely used to reveal seed protein and isozyme variation. They operate at the gene product level where the environment has very little influence (Feldman and Sears, 1981).

It is stated that germplasm collection and conservation is meaningless if it is not properly evaluated for the traits of concern (Simmands, 1979). According to him recent advances in molecular biology have allowed population geneticists to make genetic comparisons across species as well as within species because genetically heterogeneous populations produce more and stable yield than genetically homogeneous lines. Such molecular information has proven to be an important tool in systematics and in reconstructing phylogenies.

Seed protein electrophoresis is increasingly being used as an approach for species identification and as a useful tool for tracking back the profile for taxonomic and evolutionary purposes by several investigators (Luthra, 1999; Rao *et al*., 1990; Dandlani *et al*., 1994 and Ram, 1996). The patterns of SDS-electrophoresis showed the biggest difference in all types of rice grains and allowed the identification of the rice varieties (Yupsanis *et al*., 1992). Therefore SDS-PAGE of seed proteins like albumins and globulins was recommended as more suitable to distinguish among closely related varieties and pick the most diverse for breeding purposes. Ten cultivars were characterized using electrophoretic profiles of esterase, glutamate, oxaloacetate, peroxidase, amylase and lucine amino peptidase isozyme as well as SDS-PAGE of total seed proteins (Abdel Tawab *et al*., 1993). Twelve rice varieties were identified on the basis of banding pattern obtained by SDS-PAGE (Sengupta and Chattopadhyay, 2000).

The glutelin characteristics of 5 classes of rice cultivars and 6 wild rice species (*Oryza nivara*, *O. glumaepatula*, *O. latifolia*, *O. alata*, *O. stapfii* and

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Rhynchoryza subulata) were analysed by SDS-PAGE (Zhan and Line, 1991). Geographical distribution of different Brazilian rice varieties (*Oryza sativa* L.) using seed protein polymorphism was studied (Montalvan *et al*., 1995). According to them ten affinity groups were distinguished that showed several correlations with geographical distribution. This indicated that electrophoretic analysis of seed proteins can used to estimate the genetic relationship among rice varieties. Similar results were obtained by SDS-PAGE analysis of nine Japanese upland rice cultivars (Montalvan *et al*., 1998). It was concluded that electrophoretically detectable protein polymorphism in rice can indicate geographical origin as well as breeding improvement levels of cultivars.

Eight rice genotypes were characterized on the basis of electrophoresis profiles of total soluble seed proteins and found highest polymorphism among genotypes for albumin fraction (Santhy *et al*., 1998). Fifteen rice genotypes were evaluated using SDS-PAGE (Habib *et al*., 2000). Tris-soluble proteins extracted from dry seeds of the genotypes showed a total of 32 bands with certain variation in intensity and Rm values ranging from 0.33 to 1.00. Polymorphism in isoenzymes is a potential molecular marker.

Rice is most important food crop of the developing world. It occupies about 10% of the total cropped area in Pakistan and contributes 47% of food grain production. In Pakistan rice has grown on about two million hectares and is second major export crop. Therefore the purpose of the present study was to characterise the genetic diversity in rice (*Oryza sativa* L.) germplasm maintained at gene bank, PGRI, NARC, Islamabad. In order to achieve this objective the inter and intra-specific variation was ascertained and geographical distribution of genetic variability was measured by protein banding pattern using SDS-PAGE.

MATERIALS AND METHODS

Plant material: The present study was conducted at Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre, Islamabad. Twenty accessions of rice were selected on the basis of geographical distribution. These accessions were collected from the gene bank, PGRI. The plant species and geographical distribution of accessions are shown in Table 1 and seed colour, width, length and shape observed are given in Table 2.

Preparation of seed sample: For extraction of protein, individual seeds were ground to fine powder with mortar and pestle. To extract protein in 0.01 g of seed flour, 400 µl of the protein extraction buffer (0.05 M Tris-HCl, 0.2% SDS, 5 M Urea and 1% β-mercaptethanol) was added into the tube and mixed well by vortex. Then centrifuged at 15,000 rpm for 5 min. at room temperature. The extracted crude proteins were recovered as clear supernatant and stored at –20°C.

Table 1: List of rice species, their accessions and origin

Accession No.	Plant species	Origin
PAK0003071	Oryza sativa	Local
PAK0003904	*Oryza garndiglumis*	Exotic
PAK0003909	*Oryza alta*	Exotic
PAK0003919	*Oryza meridionalis*	Exotic
PAK0004036	*Oryza meridionalis*	Exotic
PAK0004042	*Oryza meridionalis*	Exotic
PAK0003918	*Oryza meridionalis*	Exotic
PAK0003924	*Oryza echingeri-uru-wee*	Exotic
PAK0004041	*Oryza echingeri-uru-wee*	Exotic
PAK0004046	*Oryza echingeri-uru-wee*	Exotic
PAK0004047	*Oryza echingeri-uru-wee*	Exotic
PAK0000411	Oryza sativa	Pak-punj
PAK0000415	*Oryza sativa*	Pak-punj
PAK0003438	*Oryza sativa*	China
PAK0003473	*Oryza sativa*	Pakistan
PAK0000516	*Oryza sativa*	Pak-punj
PAK0000681	*Oryza sativa*	Pak-sind
PAK0003459	*Oryza sativa*	Pakistan
PAK0003510	*Oryza sativa*	Pakistan
PAK0003541	*Oryza sativa*	Bangladesh

Table 2: Seed characters (colour, width, length, shape) of rice germplasm

Accession No.	Seed colour	Width (mm)	Length (mm)	Seed shape
PAK0003071	Yellowish brown	2.55	9.27	Cylindrical
PAK0003904	Light brown	2.57	10.40	Cylindrical
PAK0003909	Yellowish brown	2.77	8.78	Cylindrical
PAK0003919	Yellow	2.38	10.33	Cylindrical
PAK0004036	Dark brown	2.61	9.51	Cylindrical
PAK0004042	Light brown	3.59	7.68	Round
PAK0003918	Yellow	3.02	10.06	Cylindrical
PAK0003924	Light brown	3.48	9.96	Cylindrical
PAK0004041	Light brown	3.15	11.39	Cylindrical
PAK0004046	Light brown	3.93	7.81	Round
PAK0004047	Light brown	4.70	10.02	Cylindrical
PAK0000411	Dark brown	2.45	5.85	Round
PAK0000415	Light brown	2.33	9.01	Cylindrical
PAK0003438	Brown	2.75	9.97	Cylindrical
PAK0003473	Black	2.25	6.25	Round
PAK0000516	Blakish brown	1.90	3.50	Round
PAK0000681	Blackish brown	3.20	7.89	Round
PAK0003459	Black	3.89	9.75	Cylindrical
PAK0003510	Black	3.99	9.10	Cylindrical
PAK0003541	Yellowish brown	3.39	10.02	Cylindrical

Preparation of gel: Seed protein was analyzed through slab type SDS-PAGE followed by Laemmli (1970) using 11.25% polyacrylamide gel. Electrophoresis was carried out at 100 v for two and half-hours. In order to check reproducibility of the method two separate gels were run under similar electrophoretic conditions. After electrophoresis gels were stained with 0.2%(w/v) Coomassie brilliant blue R250 for about 1 hour. After that gels were destained over night. During staining and destaining procedures gels were subjected to constant gentle shaking on electrical shaker. After that gels were

dried using gel drying processor for about 100 min. Dried gels were analysed, scanned and photographed.

RESULTS AND DISCUSSION

Twenty accessions of rice (*Oryza sativa* L.) were analysed using SDS-PAGE. Electrophoregrams (Fig. 1B, 2B) were analysed keenly. For better illustration Zymograms (Fig. 1A, 2A) were also made on the basis of position of bands in the gel. During analysis a total of twenty-two bands were observed. Both minor and major bands were kept under consideration. With regard to the variation in banding pattern accessions were placed in clusters on the basis of presence or absence of bands. Total twelve clusters were formed (Table 3).

In the accessions of cluster 1 band numbers. 7, 10, 11, 13, 16, 18 and 20 were present and minor bands were absent. Cluster 2 included accessions in which band numbers 6, 7, 10, 14, 16 and 18 were present. In addition to that major band number 9 was present which was absent in all other accessions. In cluster 3 band numbers 6, 9, 12, 18, 19 and 21 were absent while all the other bands were present. Cluster 4 contained accessions in which band numbers 10, 11, 17, 18 and 22 were present. Accession PAK0003071 of cluster 4 showed thickness in band numbers 10 and 11 while the similar band in accession number PAK0003459 were light. Only one accession was present in cluster number 5. This accession showed only major band number 17 and minor band number 22. While all other bands are absent. In cluster 6 band numbers 9, 10, 14, 15, 16, 18, 19, 20 and 21 were absent while rest of the bands were present. Cluster 7 included accessions in which band numbers 11, 12, 13, 17, 18, 20 and 22 were present. Band numbers 6, 7 and other minor bands were absent. Four accessions were present in cluster 8 which were characterised by the presence of band number 19 which was absent in all other accessions. Band numbers 8, 11, 12, 13, 17, 18 and 22 were also present in this cluster. Only one accession was present in cluster 9 in which band numbers 9, 10, 13, 15, 16, 18, 19, 20 and 21 were absent. Cluster 10 included an accession in which only major band number 22 was present and all other bands were absent. In cluster 11 accession PAK0004047 showed presence of band numbers 5, 6 and 22 and lake major band numbers 11, 12 and other minor bands. In cluster 12 three

Table 3: Clusters based on similar protein banding patterns

Cluster No.	Accession No.	Origin
1	PAK0000411	PAK-PUNJ
1	PAK0003541	BDG
2	PAK0000415	PAK-PUNJ
2	PAK0000516	PAK-PUNJ
3	PAK0000681	PAK-PUNJ
3	PAK0003438	CHN
3	PAK0003473	PAK
3	PAK0004036	EXOTIC
4	PAK0003071	LOCAL
4	PAK0003459	PAK
5	PAK0003510	PAK
6	PAK0003904	EXOTIC
7	PAK0003909	EXOTIC
8	PAK0003918	EXOTIC
9	PAK0003924	EXOTIC
10	PAK0004042	EXOTIC
11	PAK0004047	EXOTIC
12	PAK0003919	EXOTIC
12	PAK0004046	EXOTIC
12	PAK0004041	EXOTIC

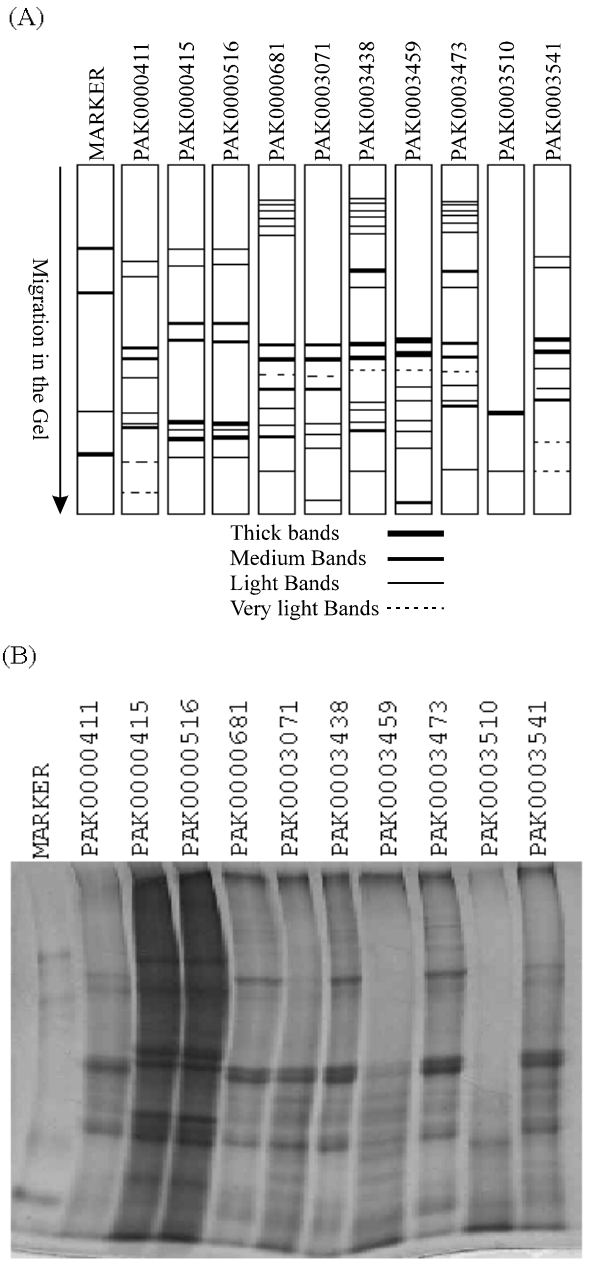

Fig. 1: Zymogram (A) and electrophoregram (B) of accessions of rice showing variation in protein banding patterns of clusters 1, 2, 3, 4 and 5

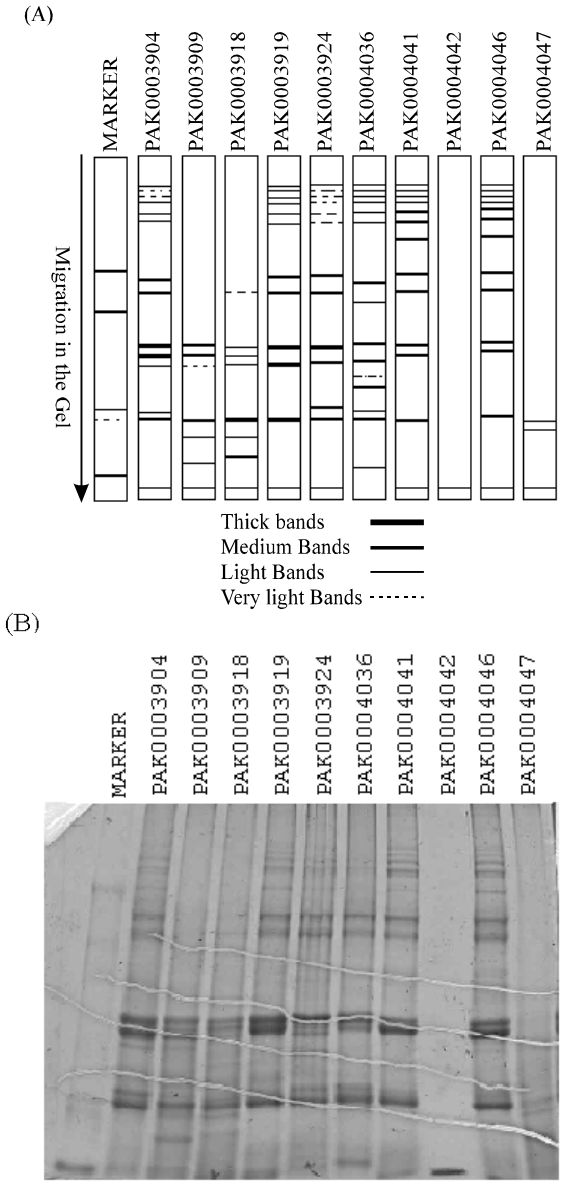

Fig. 2: Zymogram (A) and electrophoregram (B) of accessions of rice showing variation in protein spaces banding patterns of clusters 3, 6, 7, 8, 9, 10, 11 and 12

accessions PAK0003919, PAK0004041 and PAK0004046 were included in which band numbers 9, 10, 13, 14, 15, 16, 18, 19, 20 and 21 were absent while all other bands were present (Fig. 1, 2 and Table 3).

These results showed that rice germplasm investigated, possessed the considerable genetic variation which can be used for crop improvement. Similar results were obtained for rice varietal identification by SDS-PAGE (Sengupta and Chattopadhyay, 2000). In addition to inter specific variation, there was also intra-specific variation which was distributed to different geographical regions of the world.

Our findings are similar to the study of Chauhan *et al.* (2002). In twelve varieties of sorghum they also observed that varieties exhibited charactistic-banding pattern on the basis of which they can be identified accordingly. Similarly accessions of rice exhibited variation both for seed morphological traits and protein banding pattern that was correlate with phonetic distance and geographical distribution in Brazilian rice variety, using seed protein polymorphism (Montalvan *et al.*, 1995). In the present study accession from PAK-PUNJ and BDG grouped in cluster while PAK-SIND, China and exotic accessions in cluster 3. The pattern of distribution of protein bands indicated the variation within the species originated under geographical regions. Variation and geographical distribution of seed storage proteins and waxy proteins in *Setaria italica* were examined by (Afzal *et al.*, 1994). They were able to characterised total seed storage proteins in six groups, which were distributed in different geographical regions of the world.

It is evident from the results that wild accessions contained less number of bands with respect to others. It was because of the addition of new bands due to evolution and cross breeding of these varieties, leads to good quality grain. Proteins having similar motilities may show bands at the same position. Greater discriminating power can be achieved by the use of isoelectric focusing, either alone or in combination with conventional electrophoresis in a two dimensional procedure (O'Farrel, 1975). Overall results indicated that rice varieties belonging to different geographical regions are rich in genetic diversity and can be exploited to broaden the genetic base of rice improvement programs.

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