African Journal of Biotechnology Vol. 10(7), pp. 1106-1111, 14 February, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1851
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Seed storage protein polymorphism in ten elite rice (*Oryza sativa* L.) genotypes of Sindh

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Accepted 30 December, 2010

Seed protein profiling is the most promising tool in determining the molecular polymorphism and genetic homology. Seed storage proteins help in cultivar identification by avoiding the external environmental influences. Electrophoretically detectable proteins in rice grains possess the potential of characterizing the germplasms by their taxonomic and evolutionary aspects. This study was aimed at exploiting the genetic variations among ten elite rice genotypes of Sindh through electrophoretical separation of grain proteins by sodium dodecyl sulphate polyacryamide gel electrophoresis (SDS-PAGE). In conclusion, the investigation revealed negligible polymorphism, with reference to the total seed protein profiles, among the rice genotypes of Sindh, Pakistan used in this study. Hence, it is highly important to include a significant number of rice genotypes to explore their existing genotypic diversity for future rice breeding programs. The SDS-PAGE in combination with 2-D electrophoresis is further suggested for documenting contrasting variations of isoforms of protein peptides.

Key words: Seed storage proteins, polymorphism, *Oryza sativa*, Sindh, SDS-PAGE

INTRODUCTION

Rice is the most important staple food globally, and Asia contributes more than 90% of global rice production and consumption. Among the rice cultivating Asian countries, Pakistan is a major producer of many rice varieties, particularly of aromatic rice and old landraces (Rabbani et al., 2008). The crop occupies about 10% of the total crop cultivated area. Of the total value added in agriculture, it accounts for 6.1 and 1.3% to GDP. It has also been employed as a foreign exchange earning commodity (Anonymous, 2009).

In Pakistan, rice is cultivated in four distinct agro-economical zones. The first zone includes northern areas of NWFP province where rice is planted in cold areas of higher altitude and mountainous valleys. The second zone lies on the irrigated land between the rivers of Ravi and Chenab located in Punjab. The third and forth zones are comprised of the west bank of river Indus and Indus delta, respectively (Salim et al., 2003). Rice crop can potentially thrive and grow successfully in a wide range of agro-climatic conditions. However, for the last few decades, drastic changes in climatic conditions affected the cultivation and quality of rice varieties (Naz et al., 2006). Repeated use of selected rice breeding lines in various breeding programs not only limits the genetic basis but also develop susceptibility to various abiotic and biotic stresses. In these circumstances, genetic variability of existing rice germplasm should be maintained for several economical traits by conserving landrace genotypes and broadening the gene pool of aromatic rice for future breeding programs (Rabbani et al., 2008). It can be possible only by the availability of genetic variations (local and exotic germplasm) (Javid et al., 2004) and their assessment for varietal improvement (Sadia et al., 2009).

On the other hand, genotyping of different species is also necessary for characterization of different accession of crop germplasm, testing varietal purity and registration of newly developed cultivars (Chowdhury et al., 2002). Among numerous techniques available for assessing the genetic variability and relatedness among crop germplasm, seed storage protein analysis represents a valid alternative and/or improved approach to varietal identification (Mennella et al., 1999). It is a useful tool for studying

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Table 1. Details of ten elite rice genotypes used in this study.

S/N	Rice genotyoe	Source	100 grain weight (g)	Seed size
1	SADA HAYAT	Rice Research Centre, Dokri, Sindh	2.45	Bold
2	DR-82	Same	2.15	Bold
3	SHAHKAR	Same	2.37	Bold
4	IR-6	Same	2.80	Small
5	IR-8	Same	2.90	Extra bold
6	DR-92	Same	2.15	Bold
7	DR-83	Same	2.16	Small
8	DR-58	Same	2.36	Bold
9	KANWAL 95	Same	2.20	Small
10	DR-57	Same	2.28	Small

genetic diversity via sodium dodecylsulphate-poly-acrylamide gel electrophoresis (SDS-PAGE) (Sadia et al., 2009) in a short period of time (Netra and Prasad, 2007). Seed storage protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Gepts et al., 1986; Sadia et al., 2009).

Seed storage protein profiling based on SDS-PAGE can be employed for various purposes, such as characterization of germplasm (Javid et al., 2004; Iqbal et al., 2005), varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species and generation of pertinent information to complement evaluation (Sammour, 1991; Isemura et al., 2001; Ghafoor et al., 2002). There have been a substantial number of studies that have used SDS-PAGE to profile seed storage proteins in rice (Saruyama and Shinbasi, 1993; Montalvan et al., 1998; Thanh and Hirata, 2002; Netra and Prasad, 2007). Hence, it becomes highly imperative to assess genetic diversity within and between rice landraces for varietal improvement, evaluation and modification, using better methods of germplasm evaluation and charac-terization strategies. It is also required to investigate the present gene pool for selection of diverse parent cultivar and to broaden the germplasm base in the future of rice breeding programs for the sustainable management of the genetic resources of rice crops.

This study employed the use of seed storage protein polymorphism in ten rice genotypes of Sindh to assess their genetic variation and relatedness using seed storage protein polymorphism.

MATERIALS AND METHODS

Plant material

The germplasm of ten elite rice varieties were obtained from Rice Research Center, Dokri, Sindh (Table 1).

Protein extraction

For total seed protein extraction from individual seed samples, 0.5 g of each variety was taken and ground into fine powder using pestle and mortal and then, 1 ml Tris urea buffer (0.05M Tris-HCl, 2% SDS, 5M Urea, 1% β-merceptoetanol with pH 8.0) was added and the crude homogenate was centrifuged at room temperature at 15000 rpm for 10 min. The extracted protein samples were collected as supernatant and pellets were discarded and then stored at -20°C.

Protein quantification

The concentration of the extracted protein samples was determined using Bradford assay (1976) using different concentrations of the samples against the control. Relative concentrations of all samples were calculated using the formula from BSA standard chart.

Protein profiling

Protein profiling of extracted samples was analyzed through SDS-PAGE (Laemmli, 1970) using 12% polyacrylamide gel. Electrophoresis was carried out at 80 V for 3 h. A protein marker (Biorad) was loaded as standard along with the samples with equal quantities of protein (150 µl). The gels were then fixed in solution (10% acetic acid and 40% ethanol) for 15 min with constant shaking and then stained with 0.2% (w/v) Commassie brilliant blue R250 overnight on an electrical shaker. Destaining of gels was carried out for a couple of hours followed by gel preservation, scanning and photography.

Protein imaging and data analysis

Gel photographing and documentation were carried out using Biorad gel documentation system. With regard to variation in protein banding pattern, electrophoregrams of each variety was scored for the presence or absence of bands and used to construct a dendogram by the unweighed pair group method (UPAGMA). All analysis was carried out using Quantity One software (Bio-Rad).

RESULTS

In this study, the SDS-PAGE of seed proteins of ten rice genotypes was carried out to investigate the genetic diversity. Seed storage profiling showed distinct polymorphism in electrophoretic banding patterns and led to the

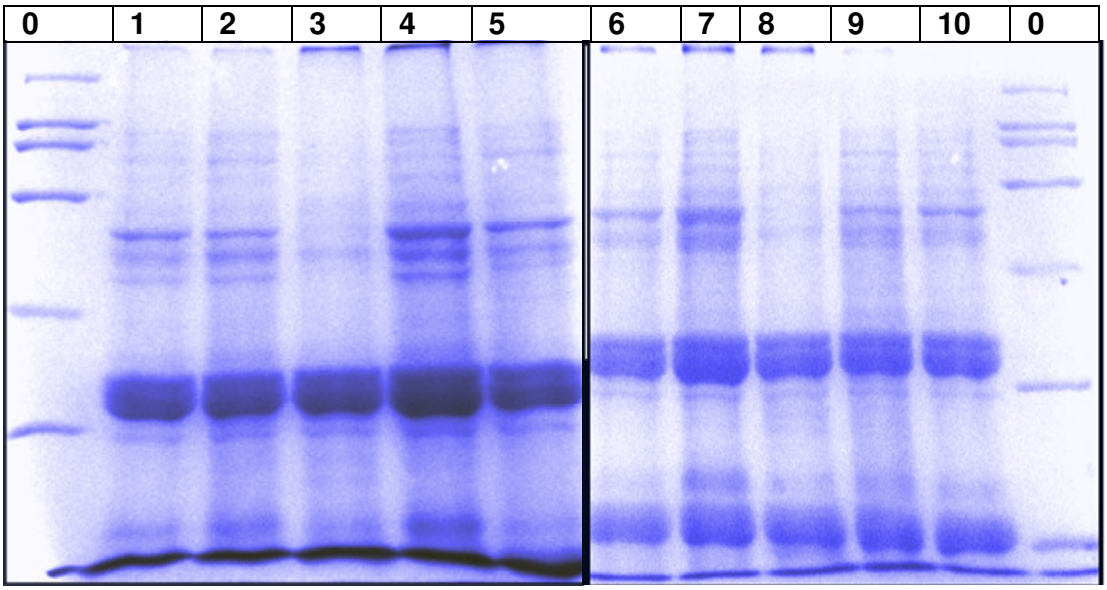

Figure 1. Seed storage proteins profiles of ten elite rice genotypes of Sindh (0, M; 1, Sada hayat; 2, DR-82; 3, Shahkar; 4, IR-6; 5, IR-8; 6, DR-5; 7, Kanwal-95; 8, DR-58; 9, Dr-83; 10, DR-92.

detection of a total of 50 polypeptide bands (Figure 1). Polymorphism was evident in all seed proteins of diverse molecular weights among all rice genotypes but major diversity was found in high molecular weight region. The molecular weights of peptides ranged from 284 to 21 kDa with the presence or absence of particular band (Table 2). Some genotypes possessed some bands which were absent in other genotypes. The rice genotypes Shahkar and DR-58 were comparatively showed more divergence than other rice genotypes. These genotypes showed subunits in narrow range of 65 to 21 kDa, while all other high molecular weight peptides were missing. All ten rice genotypes were clearly identifiable from protein banding pattern (Figure 1).

Cluster analysis, using UPGMA after quantifying the protein bands, revealed two main clusters at 70% homology (Figure 2). The data presented in Table 2 indicates the presence or absence of protein bands for each cluster. Cluster 1 was further divided into five sub clusters showing low degree of heterogeneity and close genetic proximity among genotypes. The rice genotypes IR-6, DR-82 and Kanwal 95 were grouped together into sub clusters 1 (starting from right to left). These genotypes revealed 95% homology with each other, while sub cluster 2 was grouped in the IR-8 with these three genotypes showing close proximity. Genotypes DR-82 and Sada Hayat showed 89% similarity with each other and were grouped into sub-cluster 3. Sub-cluster 4 and 5 encircled genotypes having high degree of linkage distance at 77%. Cluster 2 consisted of only two rice genotypes (Sahakar and DR-58) and showed more divergence and individuality in their banding outline comparatively from other rice germplasm (Figure 2).

DISCUSSION

Electrophoretic analysis of the seed storage proteins had direct relationship to the genetic background of the proteins that reveal genetic diversity. Such analysis can be used to certify the genetic makeup of germplasm (Javid et al., 2004; Iqbal et al., 2005). Thus, profiling of total seed storage proteins through SDS-PAGE for differentiating rice genotypes is well established (Saruyama and Shinbasi, 1993; Montalvan et al., 1998; Thanh and Hirata, 2002). It also estimates the extent of genetic variation and its geographical distribution in rice germplasm (Asghar, 2004). In the study, a wide range of protein peptides (low to high molecular weights) showed the potential for discriminating rice genotypes and can create additional variability to supplement existing germplasm. Seed storage proteins polymorphism can be used as a potential molecular marker for varietal identification and economic characterization of rice germplasm as reported earlier (Netra and Prasad, 2007).

The information gathered from cluster analysis are useful to identify contrasting parents coupled with the close genetic relatedness among various crop species for better exploitation of hybrid identification and generation of wider variability for crop improvement (Maity et al., 2009). Similarly, rice genotypes in this study showed no clear differentiation regarding origin of genotypes but exhibited homology among each other based on seed storage proteins.

In conclusion, this investigation revealed negligible polymorphism, with reference to the total seed protein profiles, among the rice genotypes of Pakistan used in this study. Hence, it is highly important to include

Table 2. Molecular weights of resolved peptides of Seed Storage Proteins of rice varieties.

S/N	Molecular weight (kD)	Sada Hayat	DR-82	SAHAKAR	IR-6	IR-8	DR-57	Kanwal-95	DR-58	DR-83	DR-92
1	284	284	…	…	…	…	…	…	…	…	…
2	282	…	…	…	…	…	…	…	…	…	…
3	277	…	277	…	…	…	…	…	…	…	…
4	115	…	…	…	…	115	…	…	…	…	…
5	114	…	114	…	…	…	…	…	…	…	…
6	111	…	…	…	111	…	…	…	…	…	111
7	108	108	…	…	…	…	…	108	…	…	…
8	106	…	…	…	…	106	…	…	…	…	…
9	98	98	…	…	…	…	98	98	…	…	…
10	97	…	…	…	…	…	…	…	…	…	97
11	94	…	…	…	94	…	…	…	…	…	…
12	91	…	…	…	…	91	…	…	…	…	…
13	87	87	87	…	…	…	…	…	…	87	87
14	86	…	…	…	…	…	…	86	…	…	…
15	85	…	…	…	…	…	85	…	…	…	…
16	77a	…	77	…	…	…	…	…	…	…	…
17	77b	…	…	…	77b	…	…	…	…	…	…
18	76	…	…	…	…	…	…	76	…	…	…
19	75	…	…	…	…	…	…	…	…	…	75
20	74	…	…	…	…	74	…	…	…	…	…
21	65	…	…	…	…	…	…	…	65	…	…
22	63	…	…	…	63	…	…	…	…	…	…
23	62	…	…	…	…	…	…	62	…	…	…
24	60	…	…	…	…	60	…	…	…	…	…
25	59	…	…	59	…	…	…	…	…	…	…
26	58	…	58	…	58	…	…	…	…	58	…
27	57	57	…	…	…	…	57	…	…	…	57
28	56	…	…	…	…	…	…	56	…	…	…
29	55	…	…	55	…	55	…	…	…	…	…
30	54	54	54	…	54	…	…	…	…	…	…
31	53	…	…	…	…	…	…	…	53	53	…
32	52	…	…	…	…	52	…	…	…	…	…
33	51	…	…	…	51	…	51	51	…	…	51
34	50	50	…	…	…	…	…	…	…	50	50
35	46	…	…	…	…	…	…	46	…	…	…
36	38	38	38	…	…	…	…	…	…	38	…
37	37	…	…	…	…	37	…	…	…	…	…
38	36	…	36	36	36	…	…	…	…	…	…
39	35	…	…	…	…	…	35	35	35	35	35
40	34	…	…	…	34	34	…	…	…	…	…
41	33	33	33	33	…	…	33	…	33	33	33
42	32	32	32	32	…	…	…	32	…	…	…
43	31	…	…	31	31	31	…	…	…	…	…
44	30	30	30	…	…	…	30	30	30	30	30
45	29	…	…	…	…	…	…	29	29	…	…
46	28	…	…	…	…	…	…	…	28	…	…
47	26	…	…	26	…	…	…	26	…	…	…
48	24	…	…	…	…	…	…	24	24	24	24
49	22	…	…	22	22	22	22	22	22	22	22
50	21	21	21	…	…	…	21	21	21	21	21
Total No. of bands		11	12	09	11	11	09	16	10	11	13

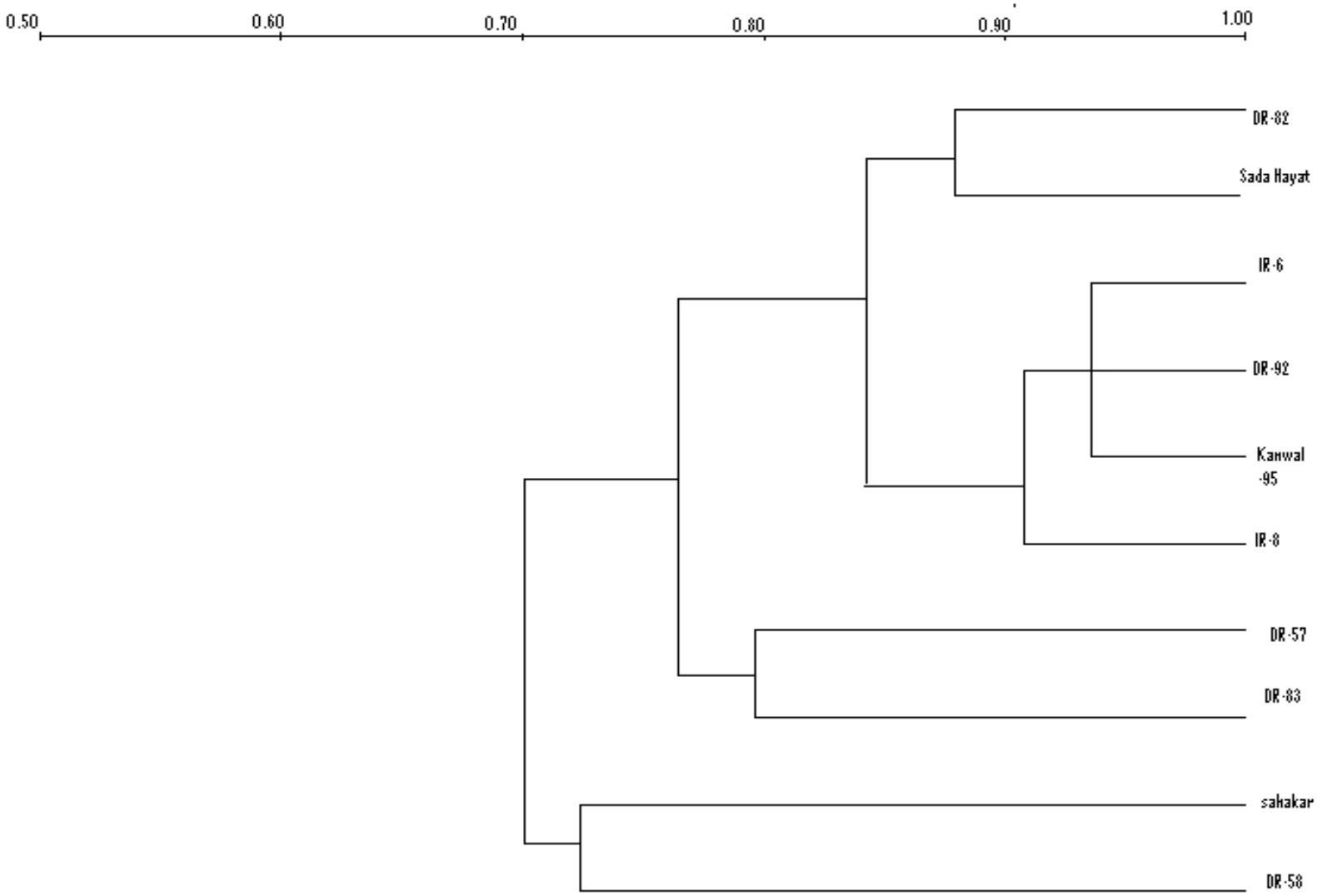

Figure 2. Dendrogram showing banding pattern associated with polymorphism in the seed storage proteins of ten elite rice genotypes of Sindh.

a significant number of rice genotypes to explore their existing genotypic diversity for future ricebreeding programs. The SDS-PAGE in combina-tion with 2-D electrophoresis is further suggested for documenting contrasting variations of isoforms of protein peptides.

ACKNOWLEDGEMENT

We are indebted to the Director, Rice Research Institute Dokri, Larkana, Sindh for the provision of good quality seeds of rice genotypes used in this study.

REFERENCES

Anonymous (2009). Economic Survey, 2008-2009. Ministry of Food, Agriculture and Livestock. Federal Bureau of Statistics. Islamabad, Pakistan.

Asghar R (2004). Inter and intra-specific variations in SDS-PAGE of total seed protein in rice (*Oryza sativa* L.) germplasm. Pak. J. Biol. Sci. 7: 139-143.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann. Biochem. 72: 248-254.

Chowdhury MA, Vandenberg B, Warkentin T (2002). Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). Euphytica, 127: 317-325.

Ghafoor A, Ahmad Z, Qureshi AS, Bashir M (2002). Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE. Euphytica, 123: 367-378.

Gepts P, Osborne TC, Rashka K, Bliss FA (1986). Phaseoline protein of Wild form variability in and landraces of the common beans (*Phaseolus vulgare*): Evidence for multiple centers of domestication. Econ. Bot. 40: 451-468.

Iqbal SH, Ghafoor A, Ayub N (2005). Relationship between SDS-PAGE markers and Ascochyta blight in chickpea. Pak. J. Bot. 37: 87-96.

Isemura T, Shiyo N, Shigeyuki M (2001). Genetic variation and geographical distribution of Azuki bean (*Vigna angularis*) landraces based on the electrophoregram of seed storage proteins. Breed. Sci. 51: 225-230.

Javid I, Ghafoor A, Anwar R (2004). Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pak. J. Bot. 36: 25-29.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Montalvan R, Ando A, Echeverrigaray S (1998). Use of seed protein polymorphism for discriminating of improvement level and geographic origin of upland rice cultivars. Genet. Mol. Biol. 21: 531-535.

Mennella G, Onofaro SV, Tonini A, Magnifico V (1999). Seed storage protein Characterization of Solanum specied and of cultivars and androgenic lines of *S. melongena* L., by SDS-PAGE and AE-hplc. Seed Sci. Technol. 27: 23-35.

Maity S, Datta AK, Chattopadhyay A (2009). Seed protein polymorphism in nine species of Jute (Corchorus, Family: *Tiliaceae*). Indian J. Sci. Technol. 2: 34-36.

Naz F, Farooq S, Arshad R, Afzaal M, Akram M (2006). Discriminating upland and lowland rice genotypes through proteomic approach. Pak. J. Bot. 38: 1731-1738.

Netra N, Prasad S (2007). Identification of rice hybrids and their parental lines based on seed, seedling characters, chemical tests and gel electrophoresis of total soluble seed proteins. Seed Sci. Technol. 35: 176-186.

Rabbani MA, Pervaiz ZH, Masood MS (2008). Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. Electron. J. Biotechnol. 11: 1-10.

Sammour RH (1991). Using electrophoretic techniques in varietal identification, biosystematic analysis, phylogenetic relations and genetic resources management. J. Islamic Acad. Sci. 4: 221-226.

Saruyama H, Shinbashi N (1993). Identification of specific proteins from seed embryos by two-dimensional gel electrophoresis for the discrimination between indica and japonica rice. Theor. Appl. Genet. 84: 947-951.

Sadia M, Malik SA, Rabbani MA, Peaece SR (2009). Electrphoretic characterization and the relationship between some brassica species. Electron. J. Biol. 5: 1-4.

Salim M, Akram M, Akhter ME, Ashraf M (2003). In: Saleem MT (ed), Rice, A production: Handbook: Pakistan Agric. Res. Council, Islamabad. p.68.

Thanh VOC, Hirata Y (2002). Seed storage protein diversity of three rice species in the Mekong Delta. Biosphere Conserv. 4: 59-67.