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STUDY OF TOTAL SEED PROTEIN PATTERN OF RICE (*ORYZA SATIVA* L.) BREEDING LINES OF PAKISTAN THROUGH SDS-PAGE

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Abstract

The rice (*Oryza sativa* L.) genotypes, including 87 breeding lines of Pakistan were evaluated for total seed storage proteins using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Total seed proteins were electrophoretically separated on 12.5% polyacrylamide gels by standard protocols. A total of 16 scorable protein bands were witnessed, of which 13 (81%) were polymorphic and 3 (19%) were monomorphic, with molecular weight ranging from 100 to 120 kDa. Two bands i.e. 5 and 6 were common in all genotypes. Similarity coefficients varied from 0.67 to 1.00. The dendrogram based on dissimilarity matrix using unweighted pair group method with arithmetic averages (UPGMA) separated all rice accessions into two main clusters i.e. I and II comprising 4 and 83 genotypes, respectively. Overall a low level of genetic diversity was detected for the total seed protein profiles, eighty seven rice breeding lines of Pakistan. Therefore, in the light of our investigations it is highly suggested to include a high number of rice genotypes for better rice breeding programs. Hence SDS-PAGE along with 2-D gel electrophoresis is further recommended. Our research will meaningfully support the cataloging, improvement, genetic assessment and conservation of rice (*Oryza sativa* L.) genotypes in Pakistan.

Introduction

Rice is one of the most important crops and a major source of nutrition for about 2.5 billion people around the globe that belongs to Poaceae family. The genus *Oryza* comprises 25 species distributed through the tropical and sub-tropical regions of the world. Rice crop can effectively survive and grow in a wide range of agro-climatic conditions (Kim *et al*., 2012). Rice plays an important role as it is the primary source of food and proteins for a major section of the world population (Khush, 1997), especially to people in the developing countries where animal proteins are expensive. In the Asian diet rice contributes almost 28-54% proteins. Rice provides 27% of the world nutritional energy and 20% of overall nutritional protein (Bashir *et al*., 2007). Rice is an excellent crop because it is one of the few plants that store and synthesize both main classes of proteins, i.e., glutelins and prolamins in sub-cellular compartments (Muench *et al*., 1998).

Currently there are 114 reported rice cultivating countries, of which 9 of the world top rice producer countries are located in Asia, while China is the largest rice producer (WWF, 2007). Rice is called the golden grain of Pakistan; about 23% of the total foreign exchange is generated by rice (Shah *et al*., 1999) and ranks third after wheat and cotton. Pakistan is a main producer of many important rice varieties, particularly of old landraces and aromatic varieties among rice growing Asian countries (Rabbani *et al*., 2008). It stands 4th in rice production in the world after China, India and Indonesia (Anon., 2007). Pakistan is famous for its basmati rice with long grain and aroma as well as for non-basmati indica varieties in the world. Forty two different rice varieties are grown in Pakistan with an average yield of 2 tons ha^{-1} (Ahmad & Awan, 2007). In Pakistan rice was cultivated on an area of 2365.3 thousand hectares during the year 2010-2011 and the total production was 4823.3 thousand tones. 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 (Anonymous, 2009).

The ability of a species to adapt to changing environment and cope with stress and its maximum utilization in breeding depends upon the genetic diversity it possesses. Wild species and landraces are the most important source of valuable genes which can be successfully used in the present day crop improvement and breeding programs to develop marvel varieties in rice that possess not only high quality and production, but also resistant to biotic and abiotic stresses (Masood *et al*., 2005). Pakistan is wealthy of rice variation due to the cultivation of diverse landraces for long time and the lack of enhanced rice varieties in the country. In almost all of the rice cultivating countries of the world green revolution resulted in the removal of traditional landraces but Pakistan is among one of the few who retained their traditional rice varieties wealth. A number of advanced rice varieties were released by Pakistan in late nineties by crossing traditional varieties and enhanced breeding lines on the basis of narrow genetic makeup (Rabbani *et al*., 2010).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a useful tool to study genetic diversity (Akbar *et al*., 2012) in a short period of time. SDS-PAGE analysis of seed storage proteins is reliable method because these proteins are not influenced by environmental conditions and are highly polymorphic (Zada *et al*., 2013). Banding patterns of proteins obtained by SDS-PAGE have been successfully employed for identification and characterization of varieties in several crop plants such as cotton, cluster bean, chickpea, soybean sesame etc. During the present study, eighty seven (87) rice breeding lines of Pakistan were used to assess diversity in total seed storage protein using SDS-PAGE.

Materials and Methods

Plant material: Plant material consisted of 87 rice breeding lines of Pakistan. These genotypes were obtained from Gene-bank of Institute of Agri-Biotechnology & Genetic Resources (IABGR), NARC, Islamabad.

Protein extraction: Whole seeds were crushed to fine powder and around 0.1 gram fine powder were put into 1.00 ml eppendorf tube. Protein extraction buffer (400µl) was mixed to flour in eppendorf tube. The extraction buffer was a mixture of 0.2%SDS, 5M Urea, 0.5M Tris-HCl (pH 8.0) and 1% 2-mercaptoethanol. Bromophenol blue was used as a dye to show the drive of polypeptides in the gel. At last samples were mixed thoroughly by centrifugation at 14,000 rpm for 10 to 12 minutes at room temperature, and kept at -4^{o}C till gel electrophoresis.

Preparation of electrophoretic gel: SDS-PAGE of total seed protein was carried out in 20% polyacrylamide using Laemmli (1970) method. Vertical slab gel was planned in a glass sandwich. The separating gel consisted of 0.135% by weight N.N-methylene-acrylamide in 0.5M Tris-HCl buffer (pH 8.8) with 0.27% SDS and 20% by weight acrylamide. The gel was polymerized by pouring 15 microlitters Tetramethylene-diamine (TEMED) and 200 microlitters ammonium per sulphate (APS). The staking gel contained 0.8% N.N-methylene-bis-acrylamide and 30% acrylamide in 0.25M Tris-HCl buffer (pH 6.8) containing 0.2% SDS. The gel was polymerized by pouring 17 microlitters Tetramethylene-diamine (TEMED) and 70 microlitters ammonium per sulphate (APS). The electrode buffer contained Tris-glycine (43.2g glycine and 9.0g Tris-HCl per 3 liters buffer solution at pH 8.9) with 3.0g SDS (0.1%). Eight microliters of sample was loaded into the stacking gel sample wells.

Electrophoresis: Electrophoresis was carried out at 80 V for around 2.30 hours till Bromophenol blue marker reached bottom of the gel. The molecular weights of parted polypeptides were determined by co-electrophoresis of molecular weight protein standards (Fermentas Life Sciences). Next electrophoresis, the gels were stained with 2% commassie blue solution for over an hour. Gels were then destained by destaining solution containing 20% (v/v) methanol, 5% (v/v) acetic acid and distilled water in the ratio of 20:5:75 (v/v) for about three hours.

Data analysis: Based on the absence or presence of protein bands, similarity index was planned for all potential pairs of polypeptide types. The score was 0 for absence and 1 for the presence of protein bands. Depending upon the outcome of electrophoretic band spectra, similarity index (s) was considered for all conceivable sets of protein type electrophoregrams by means of the following formula (Sneath & Sokal, 1973):

$$S = w/(a+b-w)$$

Results

A total of sixteen scorable protein bands were recorded among the eighty seven rice germplasm assessed (Table 1). Of these 16 protein bands, 13 (81%) were polymorphic and 3 (19%) were monomorphic. Size of the polypeptides bands produced by SDS-PAGE (dignified by Unstained Protein Molecular Weight Marker ranging from 13.5 to 116 kDa) ranged from 100 to 120 kDa. The bands 5 and 6 were common in all the genotypes, whereas band 10 was present in 2 out of 87 rice germplasm and protein bands 4, 14 and 15 were absent from only 4, 3 and 5 genotypes, respectively. The protein profile was divided into four major zones i.e., A, B, C and D. Major bands were present in the zones A and B, while bands of zones C and D were minor bands with light color (Figs. 1 & 2). It was observed that protein profile of most rice germplasm varied mostly in their minor bands as compared to the major bands. Unevenness in intensity was noticed in many protein bands that exhibited the quantity of protein peptides swelling up at a specific molecular weight.

Cluster analysis, via UPGMA procedure exposed two key clusters 1 and 2 at 69% homology (Fig. 3). The data showed in (Table 2) specifies the absence and presence of bands for diverse protein band zones. Cluster “1” was smallest cluster included 4 rice genotypes (27169, 27177, 27186 and 27195), whereas cluster “2” was the largest contained rest of the 83 rice lines and was further divided into two sub-clusters I and II. Sub-cluster I was the smallest one with only one rice genotype (27179), while sub-cluster II was the largest one with 82 rice lines presenting low level of diversity and near genetic closeness amongst rice germplasm.

Discussion

Seed storage protein profiling in rice based on SDS-PAGE have been extensively studied (Yousuf *et al*., 2003; Thanh., 2002; Parsad., 2007). Seed storage protein markers are highly polymorphic and as they work at gene product level, environmental influence on their electrophoresis pattern is limited (Gepts *et al*., 1986). Assessment of genetic diversity through SDS-PAGE is easy and cheap. Protein markers have emerged as a possible tool in studies on genetic variability and have effectively been employed for identification of varieties in a number of crop plants. Seed storage protein profiling can be used for different purposes like varietal identification, germplasm characterization, determination of phylogenetic relationship between different species and biosystematics analysis (Sammour, 1999).

Table 1. List of rice accessions used in the present study.

No.	Accession	Breeding institution/Origin	No.	Accession	Breeding institution/Origin	No.	Accession	Breeding institution/Origin
1.	27077	NARC/Islamabad	30.	27154	-	59.	27187	-
2.	27078	-	31.	27155	NARC/Islamabad	60.	27189	-
3.	27079	-	32.	27156	-	61.	27190	NARC/Islamabad
4.	27080	-	33.	27157	-	62.	27191	-
5.	27081	-	34.	27258	-	63.	27192	-
6.	27082	-	35.	27159	-	64.	27193	-
7.	27083	-	36.	27160	-	65.	27194	-
8.	27084	-	37.	27161	-	66.	27195	-
9.	27085	-	38.	27162	-	67.	27196	-
10.	72086	-	39.	27163	-	68.	27197	-
11.	27087	-	40.	27164	-	69.	27198	-
12.	27088	-	41.	27165	-	70.	27199	-
13.	72089	-	42.	27167	-	71.	27200	-
14.	27091	-	43.	27168	-	72.	27201	-
15.	27103	-	44.	27169	-	73.	27203	-
16.	27105	-	45.	27170	-	74.	27204	-
17.	27115	-	46.	27172	-	75.	27205	-
18.	27116	-	47.	27173	-	76.	27207	-
19.	27119	-	48.	27174	-	77.	27208	-
20.	27130	-	49.	27175	-	78.	27256	-
21.	27131	-	50.	27177	-	79.	27261	-
22.	27134	-	51.	27178	-	80.	27270	-
23.	27141	-	52.	27179	-	81.	27282	-
24.	27142	-	53.	27180	-	82.	27290	-
25.	27145	-	54.	27181	-	83.	27301	-
26.	27149	-	55.	27182	-	84.	27320	-
27.	27150	-	56.	27183	-	85.	27326	-
28.	27151	-	57.	27184	-	86.	27332	-
29.	27153	-	58.	27186	-	87.	27338	-

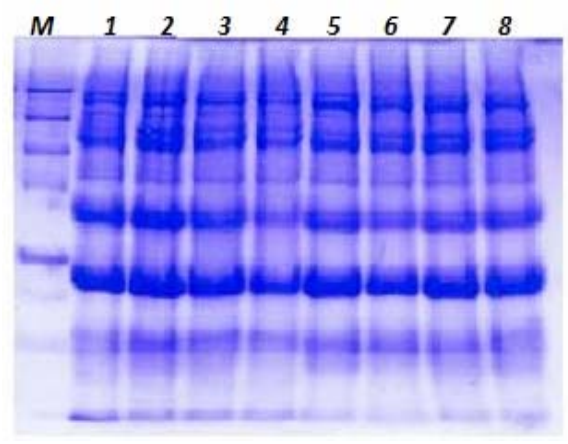

Fig. 1. Electrophoretic bands produced by SDS-PAGE of seed storage proteins of some rice genotypes. M = Protein ladder, 1 = 27077, 2 = 27078, 3 = 27082, 4 = 27083, 5 =27084, 6 = 27085,7 = 27088 and 8 = 27116.

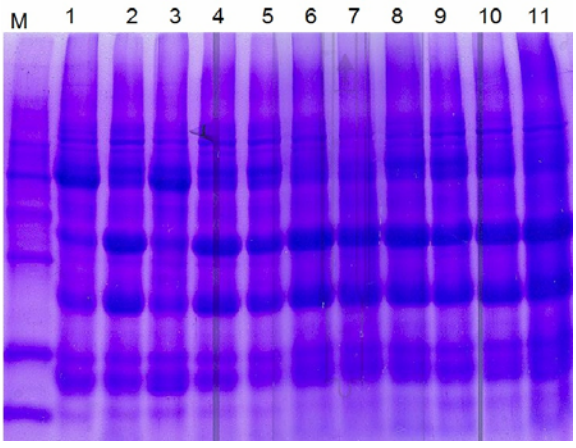

Fig. 2. Electrophoretic bands produced by SDS-PAGE of seed storage proteins of some rice genotypes. M = Protein ladder, 1 = 27085, 2 = 27086, 3 = 27087, 4 = 27088, 5 =27089, 6 = 27091,7 = 27103,8 = 27105, 9 = 27116, 10 = 27119, 11 = 27130.

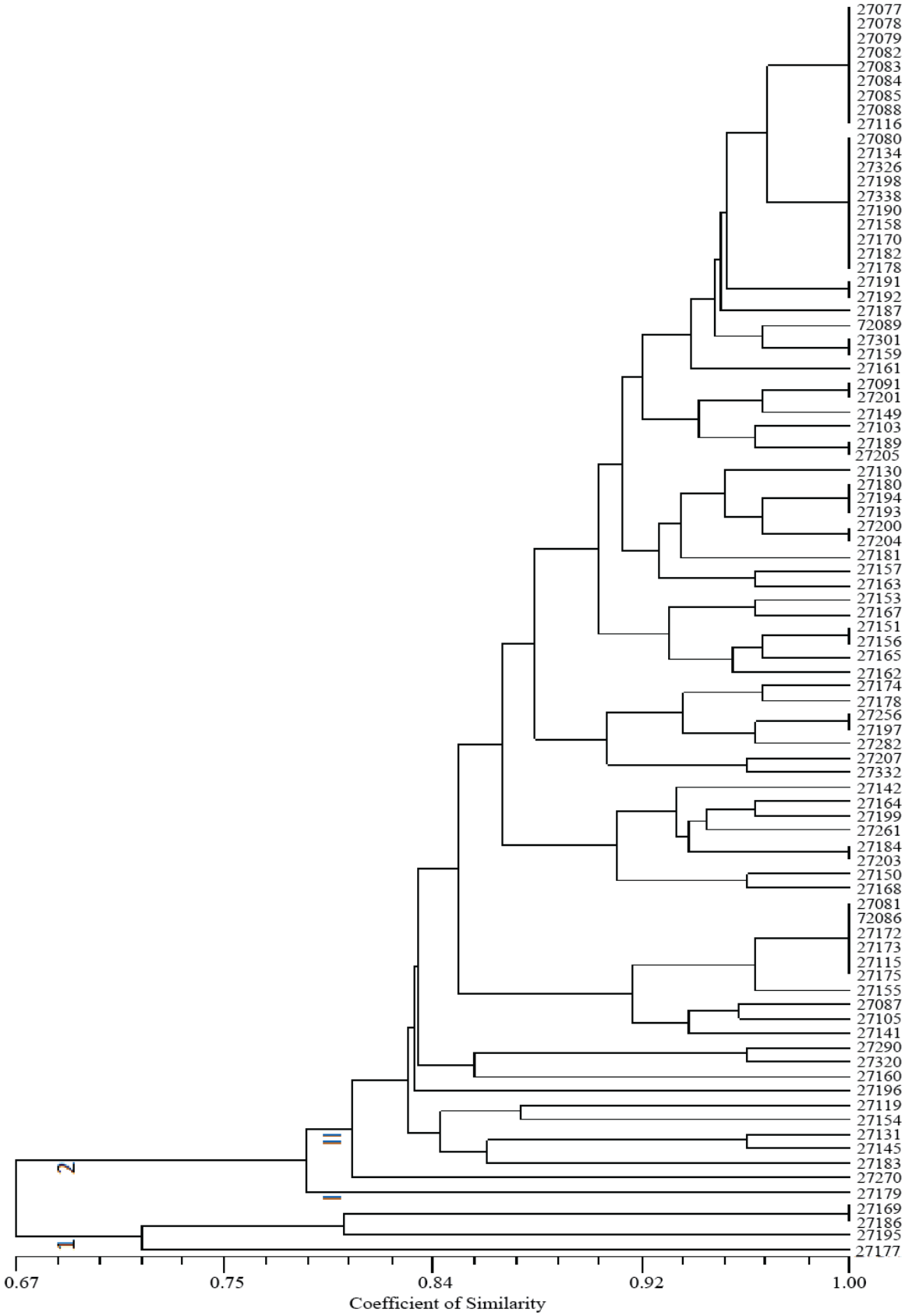

Fig. 3. Dendrogram presenting the association among Pakistani rice genotypes based on SDS-PAGE ofproteins.

Table 2. Presence and absence of protein bands in rice genotypes.

Protein zones	Protein bands	No. of genotypes	
		Presence	Absence
A	1	61	26
	2	57	30
	3	70	17
	4	83	4
	5	87	0
	6	87	0
	7	67	20
B	8	60	27
	9	69	18
	10	2	85
	11	76	11
	12	70	17
C	13	77	10
	14	84	3
D	15	82	5
	16	64	23

Our results of 87 rice (*Oryza sativa* L.) breeding lines of rice from Pakistan exposed that a narrow level of intra-specific assortment was present. Variations in major bands were existing in a few breeding lines such as 27186, 27195 and 27177, but variations in minor bands were present in maximum of rice (*Oryza sativa* L.) breeding lines. According to Ali *et al*., (2007), the equivalence in the major protein polypeptides bands among a number of genotypes states that the genes coding these proteins are conserved. Our results were supported by work of many researchers such as Das & Mukharjee (1995), De Vries *et al*., (1996) and Sultana *et al*., (2005), who reported a low to medium level of intra–specific variation for seed protein among rice (*Oryza sativa* L.) genotypes. Results of SDS-PAGE analysis showed no significance relationships among the rice breeding lines but publicized that this method offer a means for solid genotypes discrimination on the basis of genetic variation in seed protein polypeptides contrast in rice landraces of Pakistan. The matching polypeptide banding patterns revealed by the investigated rice breeding lines may be duplicated and there is a need to confirm it through high level and advanced molecular markers. In the present investigation a low level of genetic diversity noticed, so diverse genotypes based on two dimensional (2D)-electrophoresis are proposed to be achieved from a range of sources, to make a comprehensive gene pool with all-out assortment. Our research work will be helpful to make a gene bank of genetic asset of diverse rice germplasm found in Pakistan.

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