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Genetic diversity in *Oryza sativa* (L.) reported from soil erosion regions of KPK, Pakistan

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Abstract

The majority of Khyber Pakhtun Khwa (KPK) rice growing regions face problems of rapid floods each year which results soil erosion. Our current study aims to conserve the local varieties of rice growing in these floods alarming regions. About 40 genotypes were collected and evaluated through SDS-PAGE for genetic diversity. Sodium Dodicyle Sulphate Polyacrylamide Gel Electrophoresis protocol for seed storage protein profile of 40 genotypes were resolved adjusting the conditions on 12.25-15%. The experimental results from banding profile of 40 genotypes were evaluated statistically for cluster analysis and genetic disagreement. For more accuracy the electrophorogram of gels replicate were divided into three zones. The results from cluster analysis and genetic disagreement showed that present investigation was useful as provided information about 100% genetic relationships of genotypes, and with average low similarity coefficient and genetic variation of genotypes. These variations are due to the differences among various ecological conditions. However further collection and advance techniques are requires broadening the gene pool.

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Introduction

Farmers across the world face problems like soil erosion, increasing cost of chemical pesticides, weather damage recovery, the need to spray, mixing and application, yield loses and pest resistance (Khan *et al*., 2008). Soil erosion is universally recognized as a serious threat to land resources. The land resources are also being degraded in Pakistan due to erosion by water and wind. The climate of the country in arid and semi-arid areas has extreme variation in temperature. The watersheds in Upper Indus and its tributaries suffer from unfavorable soil and moisture regimes (Iqbal *et al.,* 2009).

Oryza sativa L. commonly called rice is a member of Poaceae family. *Oryza* genus contains about 25 different species which are found in the sub tropical and tropical world regions. Among these 25 species most cultivated species are *Oryza glaberrima* and Oryza sativa (Grist, 1986). Most of the world population and Asian population particularly depend upon rice as a major food crops. Rice also play pivotal role in Pakistan economy as an export item (Zahid *et al.,* 2005). Asian diet constitutes 20-40% proteins of rice (Siddiqui *et al*., 2010). Rice covers about 10% cultivated area and 17% contribution to the production of cereal grains in Pakistan (Ahmad *et al*., 2005). Pakistan grows three types of rice such as IRRI-type (grain with medium long), cold tolerant (with short and bold grain) and Basmati type (aromatic grain). Environmental conditions confined the cultivation of such varieties in specific area (Salim *et al*., 2003).

More than third world's population relies on rice even in this 21st century. Rice is cheap source of energy and constitutes 8-9% proteins (Khush, 1997). Storage proteins of rice seed based on solubility categorized into four sub families globulins (salt soluble), glutelins (soluble in dilute acid or alkali), prolamins (soluble in aqueous alcohol solutions) and albumins (water soluble) (Khan *et al*., 2010). The rice has the lowest content of seed storage proteins among cereal, accounting for 5-10% of seed weight.

Most of rice proteins are acid/alkaline soluble (glutelin) that consists of 80% total rice endosperm proteins while alcohol and salt soluble proteins (prolamin and globulin) contain relatively in low amount in rice seed endosperm (Cagampang *et al*., 1966). To solve evolutionary and taxonomic issues in many taxa of crop, seed protein profiles based on Electrophoresis technique successfully applied in identification procedure (Ferguson & Grabe 1986).

This study aims to estimates the genetic diversity of *Oryza sativa* L using SDS-PAGE technique in two habitats (irrigated and tube well water growing genotypes) KPK Pakistan. SDS-PAGE markers revealed a relatively low level of genetic variation in the studied habitats.

Materials and methods

Plant material

For evaluation of this study 40 accessions were used to asses them through SDS-PAGE markers. Among these 40 accessions, 38 accessions were collected from ecologically different regions of KPK Pakistan while two accessions were provided by the Swat research center Takhtab and KPK Pakistan. The detail is given in table 1.

Extraction of rice seed storage proteins

Fine powders of rice grain using five grains of per variety randomly were taken for protein extraction. Powder material of 0.01gram was mixed with 400 µl protein extraction buffer (PEB) in sterile Eppendorf tubes of 1.5 ml. Automated Lab-Mixer VELP scientific was used to mixed well. As a tracking dye in the gels to observe protein movements Bromophenol blue (BPB) was added. The samples were homogenized and centrifuged for 10 minutes at 12,000 rpm at room temperature. A new sterile Eppendorf tube of 1.5ml was used and the supernatant were transferred to it. Until electrophoresis samples were stored at -20°C in refrigerator.

Electrophoresis

Standard procedures as adopted by (Laemmli, 1970) were used for electrophoresis procedure.

In our current study we used slab type SDS-PAGE Model MGV-402 with resolving gel (PH 9.0, 3.0M Tris-HCl, 0.4% SDS) polyacrylamide gel 12.25-15% and stacking gel (PH 7.00, 4M Tris-HCl, 0.4% SDS) were prepared. About 17µl TEMED, 10% APS was added to polymerized. Electrode buffer solution (129M Glycine, 0.025M Tris, 0.125M SDS) was added to the apparatus in bottom pool. To avoid bubble formation Gel plates adjusted and placed in the apparatus. A solution of electrode buffer was then added in the apparatus to the top pool. Using micropipette protein sample (10 µl) of the supernatant was loaded into each wells of the gel. Uninterrupted electric supply of 80v was provided to the apparatus until we observed "Bromophenol blue" (BPB) the tracking dye in the gels plate.

Staining and destaining of gels

The gels were carefully separated and put into staining solution (40% methanol, 0.2% commassie Brilliant Blue (CBB) dissolved in 10% glacial acetic acid and water in the ratio of 10:40:50) for about one hour. A solution (20% methanol and 5% acetic acid) was used for overnight until we observed clear band of proteins. For staining and de staining shaker Model; SHO-2D was used at 40rpm speed.

Gel observation and data analysis

The gels were analyzed after washing with distilled water either on gel-drying processor for about 2-4 hours and by direct photographic method. The absence and presence of protein bands were scored using (0) for absence and (1) presence.

The intensity of bands was considered as major (Fast migration i.e. high intensity glowing bands) and minor (slow migration i.e. low intensity glowing bands). Using software STATISTICA version 7.0, UPGMA clustering method was performed to pin point relationships of the 40 rice varieties.

Results

Pattern of allele distribution

Maximum of 23 protein bands were recorded in allelic distribution pattern of 40 genotypes. Percentage variation based on allelic distribution among the different genotypes showed that high percent of variation and varieties were found such that Jolagram-V1 (91%) having high variations fallowed by 87% (Jolagram-V2), 78% (Khadagzai-V1 and Khadagzai-V2), 65% (Alladand-V2), 57% (Khadagzai-V3 and SRC-V1) and 52% (SRC-V2) respectively.

Table 1. Detail of 40 samples evaluated for SDS-PAGE genetic diversity.

S/No	Locality	Origin	Local Name	Soil nature	Hundred seed weight (g)
1	Batkhela-V1	KPK (Pak)	Garmasela	Irrigated	2.5
2	Batkhela-V2	KPK (Pak)	Begamay	Irrigated	2.8
3	Batkhela-V3	KPK (Pak)	Sarasela	Irrigated	2.6
4	Alladand-V1	KPK (Pak)	Garmasela	Irrigated	2.9
5	Alladand-V2	KPK (Pak)	Begamay	Irrigated	3.1
6	Thana-V1	KPK (Pak)	Garmasela	Irrigated	2.3
7	Thana-V2	KPK (Pak)	Begamay	Irrigated	2.6
8	Thana-V3	KPK (Pak)	Sarasela	Irrigated	2.7
9	Jalawanan	KPK (Pak)	Sarasela	Irrigated	2.8
10	Maizara	KPK (Pak)	Garmasela	Irrigated	2.2
11	Jolagram-V1	KPK (Pak)	Garmasela	Irrigated	3.6
12	Jolagram-V2	KPK (Pak)	Begamay	Irrigated	3.2
13	Badwan-V1	KPK (Pak)	Garmasela	Irrigated	2.9
14	Badwan-V2	KPK (Pak)	Begamay	Irrigated	2.6
15	Badwan-V3	KPK (Pak)	Sarasela	Irrigated	2.7
16	Khadagzai-V1	KPK (Pak)	Garmasela	Tube well	2.4
17	Khadagzai-V2	KPK (Pak)	Garmasela	Irrigated	3.2

18	Khadagzai-V3	KPK (Pak)	Begamay	Irrigated	3.4
19	Khadagzai-V4	KPK (Pak)	Sarasela	Irrigated	3.9
20	Romora-V1	KPK (Pak)	Garmasela	Irrigated	2.1
21	Romora-V2	KPK (Pak)	Begamaychina	Irrigated	2.1
22	Mayar	KPK (Pak)	Maidany	Tube well	1.9
23	Samarbagh-V1	KPK (Pak)	Maidany	Irrigated	1.8
24	Samarbagh-V2	KPK (Pak)	Maidany	Irrigated	2.1
25	Sahibabad	KPK (Pak)	Haripak	Irrigated	2.1
26	Usheraydara	KPK (Pak)	Haripak	Irrigated	2.3
27	TandkoiTopy	KPK (Pak)	Garmasela	Irrigated	3.3
28	Baghechamardan	KPK (Pak)	China/spenawreja	Irrigated	3.7
29	Muhibbandamardan	KPK (Pak)	Kinat/Indiansela	Irrigated	3.8
30	Bannu-V1	KPK (Pak)	Lawangay	Tube well	2.4
31	Bannu-V2	KPK (Pak)	Kernel	Irrigated	2.7
32	Shamozae	KPK (Pak)	Garmasela	Irrigated	3.7
33	Dadhara-V1	KPK (Pak)	Hybrid	Irrigated	3.4
34	Dadhara-V2	KPK (Pak)	China	Irrigated	3.6
35	Pangigram	KPK (Pak)	Fakhremalakand	Irrigated	2.8
36	Azematkhankale	KPK (Pak)	Fakhremalakand	Irrigated	2.5
37	Sambatswat	KPK (Pak)	China	Irrigated	3.8
38	Kokarayswat	KPK (Pak)	China	Irrigated	3.9
39	SRC-V1	KPK (Pak)	J.P-5	Irrigated	3.7
40	SRC-V2	KPK (Pak)	Basmati-385	Irrigated	3.5

Table 2. Percentage variation based on allelic distribution of 40 genotypes.

No of genotypes sharing same genetic divergence																	
Genotypes	1	1	1	1	1	1	1	2	2	2	2	4	5	5	5	6	Total=40
%age variation	91	87	65	52	43	39	22	78	57	48	19	17	30	26	13	38	Average=36

These findings are given in Table: 2.

Cluster analysis

The analysis for forty genotypes showed a similarity coefficient varying from 0.54-1.00, 0.68-1.00, 0.68-1.00 and 0.55-1.00 respectively. From dendogram dividing all the genotypes into clusters showed that two lineages are formed at 0.55 linkage distance that sorts all the genotypes into eight clusters. Similarly for Zone-I dendogram dividing into two lineages at a linkage distance of 0.68 consists of five clusters. Zone-II dendogram divides into two lineages at 0.66 constitutes of five clusters. Similarly dendogram for Zone-III sorted two lineages at a linkage distance of 0.535 and constitutes of four clusters. These results are given in figures 1-4.

From these findings most of the variations are found in cluster analysis for total polypeptides bands because of consisting more allelic information than the rest of the analysis performed for zone wise banding pattern of polypeptide bands.

Genetic distance

Analysis of genetic distance to find out genetic disagreement among the forty genotypes provide information such that 0.00% genetic disagreement existed among five comparisons while the highest genetic disagreement was recorded to be 0.87% among two comparisons as given in the table 3. Similarly forty one comparisons in Zone-I was found to have 0.00% and sixteen comparisons 0.09% genetic disagreement being minimum and maximum values.

Table 3. Genetic disagreement based on total polypeptide bands of seed storage protein profile of 40 rice genotypes.

Locality	Batkhela v1	Batkhela v2	Batkhela v3	Alladand v1	Alladand v2	Thana v1	Thana v2	Thana v3	Jalawanan	Maizara	Jolagram v1	Jolagram v2	Badwan v1	Badwan v2	Badwan v3	Khadagzai v1	Khadagzai v2	Khadagzai v3	Khadagzai v4	Ramora v1	Ramora v2	Mayar	Samarbagh v1	Samarbagh v2	Sahibabad	Usheraydara	TandkoiTopy	BaghechaMardan	Muhibbandamardan	Bannu v1	Bannu v2	Shamozae	Dadhara v1	Dadhara v2	pangigram	Azeemaykhankale	Sambatswat	Kokaraiswat	SRC v1	SRC v2
Batkhela v1	0.00																																						
Batkhela v2	0.00	0.00																																					
Batkhela v3	0.22	0.22	0.00																																				
Alladand v1	0.43	0.43	0.22	0.00																																			
Alladand v2	0.70	0.70	0.48	0.26	0.00																																		
Thana v1	0.09	0.09	0.22	0.43	0.61	0.00																																	
Thana v2	0.04	0.04	0.26	0.48	0.65	0.04	0.00																																
Thana v3	0.04	0.04	0.26	0.48	0.65	0.04	0.00	0.00																															
Jalawanan	0.39	0.39	0.26	0.13	0.39	0.39	0.43	0.43	0.00																														
Maizara	0.17	0.17	0.22	0.35	0.61	0.17	0.22	0.22	0.30	0.00																													
Jolagram v1	0.78	0.78	0.65	0.43	0.26	0.78	0.83	0.83	0.48	0.61	0.00																												
Jolagram v2	0.83	0.83	0.61	0.39	0.22	0.83	0.87	0.87	0.52	0.65	0.13	0.00																											
Badwan v1	0.17	0.17	0.30	0.52	0.78	0.26	0.22	0.22	0.48	0.35	0.78	0.74	0.00																										
Badwan v2	0.39	0.39	0.17	0.30	0.57	0.39	0.43	0.43	0.26	0.39	0.57	0.52	0.22	0.00																									
Badwan v3	0.35	0.35	0.30	0.43	0.61	0.35	0.39	0.39	0.39	0.35	0.61	0.57	0.17	0.13	0.00																								
Khadagzai v1	0.74	0.74	0.52	0.39	0.22	0.74	0.78	0.78	0.52	0.65	0.13	0.09	0.65	0.43	0.48	0.00																							
Khadagzai v2	0.74	0.74	0.52	0.39	0.22	0.74	0.78	0.78	0.52	0.65	0.13	0.09	0.65	0.43	0.48	0.00	0.00																						
Khadagzai v3	0.52	0.52	0.30	0.43	0.43	0.52	0.57	0.57	0.48	0.43	0.35	0.30	0.43	0.22	0.26	0.22	0.22	0.00																					
Khadagzai v4	0.35	0.35	0.30	0.43	0.61	0.35	0.39	0.39	0.39	0.35	0.61	0.57	0.17	0.13	0.00	0.48	0.48	0.26	0.00																				
Ramora v1	0.30	0.30	0.26	0.39	0.57	0.30	0.35	0.35	0.43	0.30	0.57	0.52	0.22	0.17	0.04	0.43	0.43	0.22	0.04	0.00																			
Ramora v2	0.22	0.22	0.26	0.39	0.65	0.30	0.26	0.26	0.35	0.39	0.74	0.70	0.13	0.26	0.30	0.61	0.61	0.48	0.30	0.35	0.00																		
Mayar	0.22	0.22	0.26	0.48	0.74	0.30	0.26	0.26	0.43	0.30	0.83	0.78	0.13	0.26	0.22	0.70	0.70	0.48	0.22	0.26	0.17	0.00																	
Samarbagh v1	0.30	0.30	0.35	0.48	0.65	0.39	0.35	0.35	0.52	0.39	0.74	0.70	0.22	0.35	0.30	0.61	0.61	0.48	0.30	0.35	0.26	0.09	0.00																
Samarbagh v2	0.39	0.39	0.43	0.39	0.57	0.48	0.43	0.43	0.43	0.48	0.65	0.61	0.30	0.43	0.39	0.52	0.52	0.57	0.39	0.43	0.26	0.17	0.09	0.00															
Sahibabad	0.39	0.39	0.43	0.48	0.65	0.48	0.43	0.43	0.52	0.48	0.65	0.70	0.30	0.43	0.39	0.61	0.61	0.65	0.39	0.43	0.26	0.17	0.17	0.17	0.00														
Usheraydara	0.30	0.30	0.35	0.48	0.65	0.39	0.35	0.35	0.43	0.39	0.74	0.70	0.22	0.35	0.30	0.61	0.61	0.48	0.30	0.35	0.26	0.09	0.09	0.09	0.26	0.00													
TandkoiTopy	0.35	0.35	0.39	0.43	0.70	0.43	0.39	0.39	0.48	0.43	0.70	0.74	0.26	0.39	0.35	0.65	0.65	0.61	0.35	0.39	0.22	0.13	0.13	0.13	0.04	0.22	0.00												
BaghechaMardan	0.26	0.26	0.30	0.52	0.70	0.35	0.30	0.30	0.48	0.35	0.78	0.74	0.17	0.30	0.26	0.65	0.65	0.43	0.26	0.30	0.22	0.04	0.13	0.22	0.22	0.13	0.17	0.00											
Muhibbandamardan	0.17	0.17	0.30	0.52	0.70	0.26	0.22	0.22	0.48	0.35	0.78	0.74	0.09	0.30	0.26	0.65	0.65	0.43	0.26	0.30	0.13	0.13	0.22	0.30	0.30	0.22	0.26	0.09	0.00										
Bannu v1	0.30	0.30	0.35	0.48	0.65	0.39	0.35	0.35	0.43	0.39	0.65	0.61	0.22	0.26	0.30	0.52	0.52	0.30	0.30	0.35	0.26	0.26	0.26	0.35	0.43	0.26	0.39	0.30	0.22	0.00								
Bannu v2	0.39	0.39	0.52	0.48	0.65	0.48	0.43	0.43	0.43	0.48	0.65	0.52	0.22	0.35	0.30	0.61	0.61	0.48	0.30	0.35	0.35	0.35	0.43	0.43	0.52	0.35	0.48	0.30	0.22	0.35	0.00							
Shamozae	0.22	0.22	0.35	0.57	0.83	0.30	0.26	0.26	0.52	0.39	0.83	0.70	0.04	0.26	0.22	0.70	0.70	0.48	0.22	0.26	0.17	0.17	0.26	0.35	0.35	0.26	0.30	0.22	0.13	0.26	0.17	0.00						
Dadhara v1	0.22	0.22	0.35	0.57	0.83	0.30	0.26	0.26	0.52	0.39	0.83	0.70	0.04	0.26	0.22	0.70	0.70	0.48	0.22	0.26	0.17	0.17	0.26	0.35	0.35	0.26	0.30	0.22	0.13	0.26	0.17	0.00	0.00					
Dadhara v2	0.39	0.39	0.43	0.39	0.65	0.48	0.43	0.43	0.43	0.57	0.65	0.52	0.22	0.35	0.39	0.52	0.52	0.57	0.39	0.43	0.17	0.35	0.35	0.26	0.35	0.35	0.30	0.39	0.30	0.43	0.26	0.17	0.17	0.00				
pangigram	0.30	0.30	0.43	0.48	0.74	0.39	0.35	0.35	0.43	0.48	0.74	0.61	0.13	0.26	0.22	0.61	0.61	0.48	0.22	0.26	0.17	0.26	0.35	0.35	0.35	0.35	0.30	0.30	0.22	0.26	0.17	0.09	0.09	0.17	0.00			
Azeemaykhankale	0.35	0.35	0.39	0.43	0.70	0.43	0.39	0.39	0.39	0.43	0.70	0.57	0.17	0.30	0.35	0.65	0.65	0.52	0.35	0.39	0.13	0.30	0.39	0.39	0.39	0.39	0.35	0.35	0.26	0.39	0.22	0.13	0.13	0.13	0.13	0.00		
Sambatswat	0.43	0.43	0.57	0.43	0.61	0.52	0.48	0.48	0.48	0.52	0.61	0.48	0.26	0.48	0.43	0.57	0.57	0.61	0.43	0.48	0.30	0.39	0.39	0.30	0.39	0.39	0.35	0.35	0.26	0.48	0.13	0.22	0.22	0.13	0.22	0.17	0.00	
Kokaraiswat	0.52	0.52	0.39	0.35	0.52	0.52	0.57	0.57	0.30	0.52	0.52	0.39	0.35	0.30	0.43	0.48	0.48	0.43	0.43	0.48	0.30	0.48	0.57	0.48	0.57	0.48	0.52	0.43	0.35	0.48	0.22	0.30	0.30	0.22	0.30	0.17	0.17	0.00
SRC v1	0.61	0.61	0.48	0.35	0.43	0.61	0.65	0.65	0.39	0.61	0.43	0.30	0.43	0.39	0.43	0.39	0.39	0.43	0.43	0.48	0.39	0.57	0.57	0.48	0.57	0.57	0.52	0.52	0.43	0.57	0.30	0.39	0.39	0.22	0.39	0.26	0.17	0.09	0.00	
SRC v2	0.57	0.57	0.43	0.39	0.48	0.57	0.61	0.61	0.43	0.57	0.48	0.35	0.39	0.35	0.39	0.43	0.43	0.39	0.39	0.43	0.43	0.52	0.52	0.52	0.61	0.52	0.57	0.48	0.39	0.52	0.26	0.35	0.35	0.26	0.43	0.30	0.22	0.13	0.04	0.00

Zone-II was found to have 0.00% genetic disagreement for seventy six comparisons and a maximum of 0.86% genetic disagreement for nine comparisons. Similarly Zone-III was found to have a minimum of genetic disagreement 0.00% for one.

Average genetic distance

Using different parameters such as average genetic distance of each genotype, zone wise average genetic distance of the all genotypes, total average genetic distance of genotypes, zone wise total average genetic distance result were tabulated in the given table 4.

Table 4. Average genetic distance for different parameters.

Average genetic distance of each genotype	Locality	Total A*	1-10B*	11-17B*	18-23B*
	Batkhela-V1	0.35	0.37	0.28	0.42
	Batkhela-V2	0.35	0.37	0.28	0.42
	Batkhela-V3	0.35	0.42	0.28	0.32
	Alladand-V1	0.41	0.52	0.34	0.32
	Alladand-V2	0.57	0.63	0.57	0.45
	Thana-V1	0.39	0.38	0.28	0.55
	Thana-V2	0.39	0.37	0.28	0.55
	Thana-V3	0.39	0.37	0.28	0.55
	Jalawanan	0.41	0.49	0.3	0.42
	Maizara	0.4	0.37	0.43	0.42
	Jolagram-V1	0.6	0.63	0.66	0.47
	Jolagram-V2	0.56	0.63	0.66	0.31
	Badwan-V1	0.3	0.37	0.25	0.23
	Badwan-V2	0.33	0.45	0.25	0.23
	Badwan-V3	0.33	0.44	0.25	0.23
	Khadagzai-V1	0.52	0.63	0.6	0.23
	Khadagzai-V2	0.52	0.63	0.6	0.23
	Khadagzai-V3	0.43	0.49	0.5	0.23
	Khadagzai-V4	0.33	0.44	0.25	0.23
	Ramora-V1	0.34	0.44	0.28	0.23
	Ramora-V2	0.31	0.4	0.25	0.23
	Mayar	0.32	0.36	0.34	0.23
	Samarbagh-V1	0.36	0.39	0.41	0.23
	Samarbagh-V2	0.37	0.43	0.41	0.23
	Sahibabad	0.41	0.48	0.34	0.38
	Usheraydara	0.35	0.39	0.41	0.23
	TandkoiTopy	0.38	0.41	0.34	0.38
	Baghechamardan	0.34	0.36	0.39	0.23
	Muhibbandamardan	0.31	0.37	0.3	0.23
	Bannu -V1	0.37	0.42	0.41	0.23
	Bannu –V2	0.37	0.42	0.35	0.31
	Shamozae	0.32	0.37	0.25	0.31
	Dadhara-V1	0.32	0.37	0.25	0.31
	Dadhara-V2	0.35	0.46	0.25	0.31
	Pangigram	0.33	0.41	0.25	0.31
	Azematkhankale	0.35	0.4	0.3	0.31
	Sambatswat	0.38	0.44	0.35	0.31
	Kokarayswat	0.39	0.47	0.35	0.31
	SRC-V1	0.42	0.54	0.35	0.31
	SRC-V2	0.42	0.53	0.35	0.31
		0.39C*	0.45D*	0.36D*	0.32D*

Key: Average genetic distance of each genotype A*, zone wise average genetic distance of the all genotypes B*, total average genetic distance of genotypes C*, zone wise total average genetic distance D*.

The first parameter provides information such that Khadagzai-V2, Khadagzai-V1, Jolagram-V2, Jolagram-V1 and Alladand-V2 showed high genetic disagreement of 0.52, 0.52, 0.56, 0.57 and 0.60 respectively.

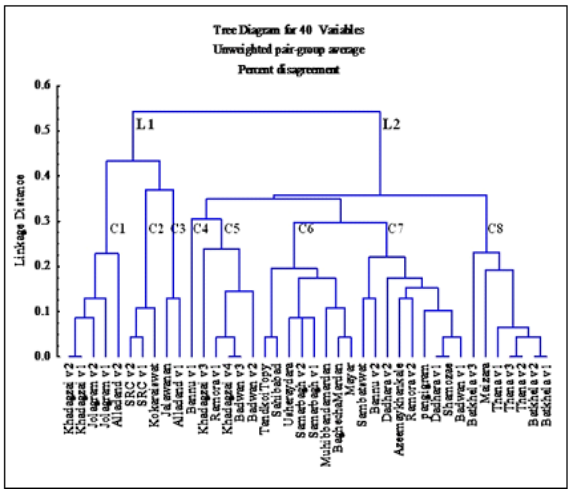

Fig. 1. Cluster analysis of 40 accessions based on total polypeptide bands.

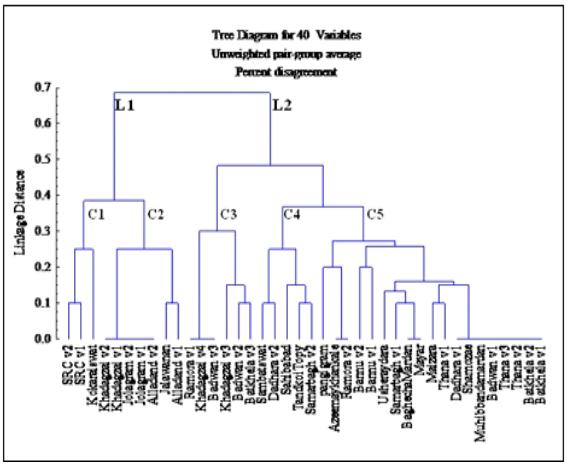

Fig. 2. Cluster analysis of 40 accessions based on zone-I.

The second parameter showed that high genetic disagreement recorded for genotype Alladand-V1 0.52 in Zone-I, 0.63 and 0.57 for Alladand-V2, 0.66 for Jolagram-VI and Jolagram-V2 in Zone-II. Third parameter showed a significant 0.39% genetic disagreement. Fourth parameter showed 0.32%, 0.0.36% and 0.45% genetic disagreement for Zone-I, Zone-II and Zone-III respectively.

Discussion

SDS-PAGE analysis is one of the promising tools to resolve evolutionary and taxonomic problems (Ghafoor, 1999). Seed storage proteins are mostly independent of environmental changes therefore SDS-PAGE technology is considered as reliable technique (Iqbal *et al.*, 2005). Electrophoresis of proteins is a powerful tool to study population genetics among most of the taxa (Parker *et al.*, 1998). To assess accurate indices of genetic diversity biochemical markers are widely used (Rabbani *et al.*, 2001).

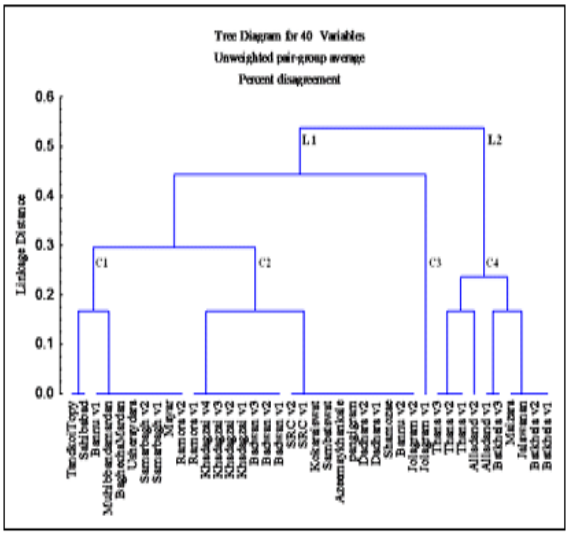

Fig. 3. Cluster representation of 40 accessions based on zone-II.

Using SDS-PAGE technique for studying 15 genotypes of rice total of 32 bands was scored (Habib *et al.*, 2000). Minimum of twelve and maximum of 17 bands were observed using SDS-PAGE technique (Sharief *et al.*, 2005). According to our banding profile recorded among the 40 genotypes of rice a total of 23 bands were scored in the present study.

Electrophorogram can divides into different zones for specific germplasm to obtain good results for the picture of genetic diversity (Ghafoor *et al.*, 2002 & Nisar *et al.*, 2009). Gel replicates in this study were divided into three prominent zones.

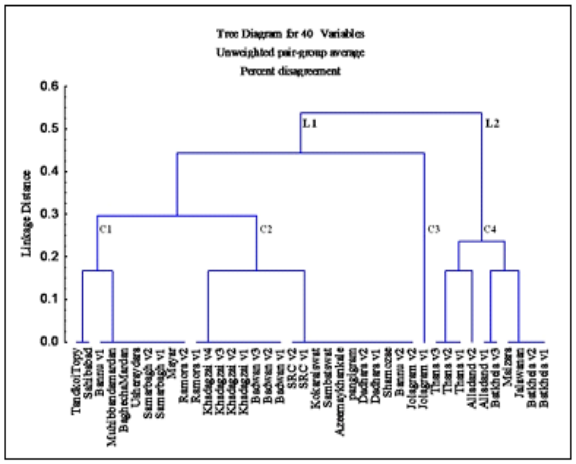

Fig. 4. Cluster representation of 40 accessions based on zone III.

Similarity coefficient varying from 0.54-1.00, 0.68-1.00, 0.68-1.00 and 0.55-1.00 respectively based on cluster analysis performed for total and zone wise banding pattern for forty genotypes showed consistency with those recorded by Sultana *et al.*, (2005), Nisar *et al.*, (2009), Buyukunal Bal & Bay (2010) and Inamullah *et al.*, (2010).

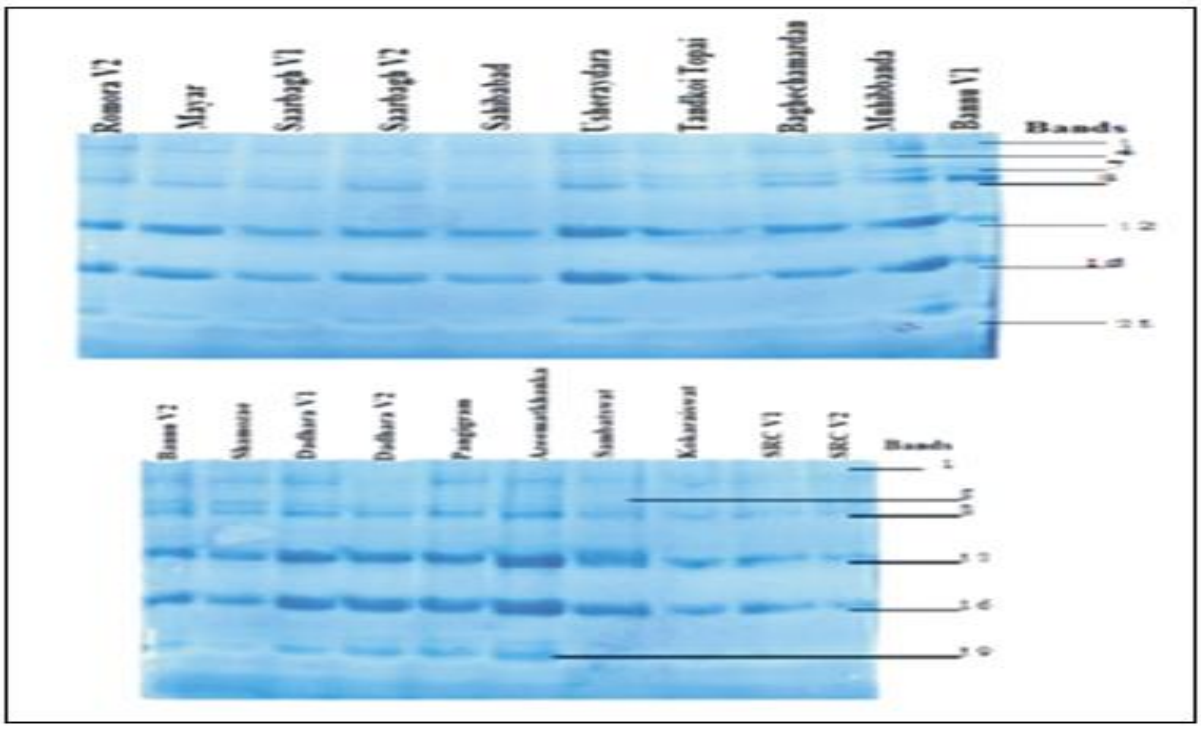

Fig. 5. Seed storage protein profile of rice genotypes.

Maximum of 0.80% genetic disagreement recorded for eleven rice varieties locally grown in Pakistan (Inamullah *et al.*, 2010). Maximum of 0.93% genetic disagreement recorded in rice varities of Turish rice (Buyukunak Bal & Bay 2010). In the current study of forty rice genotypes locally grown in KPK, Pakistan maximum of 0.90% genetic disagreement was recorded. Results reported by Sultana *et al.*, 2005 also show similar relation to the current study.

Conclusion

This study provides information for conservation of some variable genotypes like Alladand-V1, Thana-V1, Alladand-V2, Thana-V2 Khadagzai-V1, SRV1 and SRCV2 due to low similarity coefficient. These varieties are endangered in nature due to floods wash away most of these areas each year. Moreover most of the local cultivated genotypes have been replaced by commercially growing cultivars thus to maintain the back up of these varieties in the form of gene bank is necessary for these local cultivated genotypes.

It is recommended that the collection made from different regions provided high degree of variation, which needed further study to get genetic diversification in these studied accessions for new varieties and to improve their genetic traits for further research programs.

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