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Genetic Variability Assessment of Maize (*Zea mays* L.) Germplasm Based on Total Seed Storage Proteins Banding Pattern Using SDS-PAGE

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Abstract:

The characterization of genetic diversity of inbred lines is the basic prerequisite for the successful breeding programs of maize like other crops. In the present investigation 73 genotypes of maize from China, Japan and Pakistan were characterized for the total seed storage proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The total protein bands were separated on 12% polyacrylamide gel using standard protocols. A total of 18 protein bands were recorded. Among these 7 (39%) were monomorphic and 11 (61%) polymorphic, with molecular weight varied from 10kDa to 122kDa.Coefficient of similarity matrix varied between .84 and 1. The dendrogram resulted by applying UPGMA clustering method separated the whole genotypes into two main groups; I and II. Cluster I

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comprised of 66 genotypes including Agaiti-2002 and Sadaf, while cluster II possessed 7 genotypes including Sahiwal-2002. This biochemical marker based analysis resulted in over all a low level of polymorphism both in indigenous and exotic accessions. It is concluded from the present study that more diverged intra specific genotypes could be studied by using the SDS-PAGE technique. It seems to be effective tool for the maintenance, evaluation and utilization of maize germplasm which is the only source to be utilized for the development of new varieties during breeding programs.

Key words: Genetic varbiality; Maize; seed proteins; dendrogram; polymorphism; SDS-PAGE.

Introduction

It is extremely vital to have enough knowledge about the important biological phenomena at molecular level that occur in plants to effectively conserve, manage and utilize plant genetic resources. To improve crop genetic resources it is pivotal to know about the genetic diversity they possess. For this purpose there is continuous study of wild relatives and traditional varieties through latest breeding techniques. During all these efforts the extent of genetic diversity is detected at some level to frame the resistant and high yielding varieties (Mondini *et al*., 2009).

Genetic variations are any discrepancies among individuals at nucleotides, genes, chromosomes or whole genomes level (Williams and Humphries, 1996). Populations having individuals with more allelic variation will cope with new pressures and survive for more generation. Genetic diversity is directly related to the basic requirements generally of all organisms including human, especially in the context of food security and energy availability for the rapidly increasing

human population. It is alarming issue in under developed and developing countries like Pakistan. To cope with these and many other challenges the development of productive maize varieties for ethanol production as alternative fuel and dual purpose varieties and hybrids with favorable trait(s) is needed. For this purpose the knowledge of genetic diversity, to elucidate the degree of genetic variability in the base material is extremely important (Sumalini and Manjulatha, 2012).

The usefulness of broad genetic makeup is quite evident as narrow genetic makeup is always suffering from the threat of genetic vulnerability to different stresses (Masood *et al*, 2005). That is the reason that the estimation of genetic diversity of maize germplasm not only needed for the planning of successful breeding programs but also for its effective conservation.

Different methodologies are followed to study the genetic diversity in maize germplasm. In the description of genetic characterization of plant genetic resources, the morphological characterization is considered the initial step (Smith and Smith, 1989) but do not give consistent and reliable results because of the possibility of affecting by different environmental factors, pleiotropism, late onset of certain morphological traits, low polymorphism and manmade mistakes during handling. After the discovery that proteins and enzymes are actually the results of information stored in genes, they are used as biochemical markers in the form of isozymes and other proteins to analyze the genetic diversity of populations (Scandalios, 1969, Brown, 1978, Hamrick *et al*., 1979). The biochemical study particularly proteins (Wallace *et al*., 1990) is based on the separation of protein in to specific banding pattern. The cereal grain proteins have been investigated for more than 268 years (Beccari, 1745). Osborne (1907) and later on many others studied cereal proteins. Presently biochemical markers are extensively used to make assessment of the genetic diversity. Its preference over the

agro-morphological traits is because of the almost truly coping the genetic information (Perry and McIntosh, 1991).

In protein fingerprinting the proteins are extracted from different parts of plant i.e. leaves, shoots, seeds etc. Their electrophoresis is done through sodium dodecyl sulphate polyacrylamide gel. The electrophoresis of seed storage proteins is valuable approach for the identification and characterization of various cultivars as it is reproducible, rapid and cost effective (Laemmli, 1970). Hybrids and lines can be differentiated by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (Koranyi, 1989). The maize genetic purity and verity identification can be carried out effectively through sodium dodecyl sulphate polyacrylamide gel electrophoresis (Khan *et al*., 2013). Protein subunits in the polyacrylamide gel translate their genetic background through which the wild or newly made cereals from wild parents can be investigated (Gorinstein *et al*., 1999). For characterizing the extent of genetic diversity in maize seed storage proteins, the sodium dodecyl sulphate polyacrylamide gel electrophoresis has proved effective (Paulis *et al*., 1975). It has been studied that besides physiochemical and molecular study, the SDS-PAGE is fruitful to study the genetic variability in seed storage proteins (Zeb *et al*., 2006). During the present investigation 73 genotypes of maize were evaluated to fingerprint the diversity and differentiation in total seed storage proteins. The objectives were to find out the level of genetic variability present in maize germplasm by using the electrophoretic profiles of total seed proteins with different molecular weights through SDS- PAGE.

Materials and Methods

Plant material: Plant material consisted of 73 genotypes of maize including 70 collections and three commercial varieties named Agaiti-2002, Sadaf and Sahiwal-2002 of maize (Table 2). Seed material obtained from national gene bank of Plant

Genetic Resource Institute (PGRI), National Agricultural Reaserch Center (NARC), Islamabad.

Table 2: List of maize accessions used during the present study and their origin.

No.	Accession	Collecting Organization	Origin	No.	Accession	Collecting Organization	Origin
1	14862	MSM/NARC	Pakistan	38	15027	PARC/IBPGR	Pakistan
2	14878	PARC/SVP	Pakistan	39	15030	CHINA	China
3	14880	PARC/SVP	Pakistan	40	15043	CHINA	China
4	14883	PARC/SVP	Pakistan	41	15044	CHINA	China
5	14884	PARC/SVP	Pakistan	42	15047	PARC/CHINA	Pakistan
6	14886	PARC/SVP	Pakistan	43	15050	PARC/CHINA	Pakistan
7	14894	PARC/SVP	Pakistan	44	15074	PARC/IBPGR	Pakistan
8	14895	PARC/SVP	Pakistan	45	15090	PARC/IBPGR	Pakistan
9	14898	PARC/SVP	Pakistan	46	15092	PARC/IBPGR	Pakistan
10	14899	PARC/SVP	Pakistan	47	15096	PARC/IBPGR	Pakistan
11	14900	PARC/SVP	Pakistan	48	15097	PARC/IBPGR	Pakistan
12	14903	PARC/SVP	Pakistan	49	15106	PARC/IBPGR	Pakistan
13	14904	PARC/SVP	Pakistan	50	15107	PARC/IBPGR	Pakistan
14	14906	PARC/SVP	Pakistan	51	15108	PARC/IBPGR	Pakistan
15	14907	PARC/SVP	Pakistan	52	15117	PARC/IBPGR	Pakistan
16	14909	PARC/SVP	Pakistan	53	15118	PARC/IBPGR	Pakistan
17	14911	PARC/SVP	Pakistan	54	15145	PARC/KUJ	Pakistan
18	14912	PARC/SVP	Pakistan	55	15149	PARC/KUJ	Pakistan
19	14913	PARC/SVP	Pakistan	56	15160	UAF/FAISALABAD	Pakistan
20	14914	PARC/SVP	Pakistan	57	15162	UAF/FAISALABAD	Pakistan
21	14915	PARC/SVP	Pakistan	58	15168	PARC/NIAR	Pakistan
22	14924	PARC/SVP	Pakistan	59	15172	PARC/NIAR	Pakistan
23	14954	PARC/IBPGR	Pakistan	60	15209	PARC/NIAR	Pakistan
24	14959	PARC/IBPGR	Pakistan	61	15215	PARC/NIAR	Pakistan
25	14979	PARC/IBPGR	Pakistan	62	15227	PARC/JICA	Pakistan
26	14987	PARC/IBPGR	Pakistan	63	15228	PARC/JICA	Pakistan
27	14993	PARC/IBPGR	Pakistan	64	15231	PARC/JICA	Pakistan
28	15000	PARC/IBPGR	Pakistan	65	15232	PARC/JICA	Pakistan
29	15007	PARC/IBPGR	Pakistan	66	15236	PARC/JICA	Pakistan
30	15009	PARC/IBPGR	Pakistan	67	15244	PARC/JICA	Pakistan
31	15011	PARC/IBPGR	Pakistan	68	15250	PARC/JICA	Pakistan
32	15012	PARC/IBPGR	Pakistan	69	15258	KAES-MAFF	Japan
33	15013	PARC/IBPGR	Pakistan	70	15262	KAES-MAFF	Japan
34	15014	PARC/IBPGR	Pakistan	71	Agaiti – 2002	MMRI,Yousafwala	Pakistan
35	15020	PARC/IBPGR	Pakistan	72	Sadaf	MMRI,Yousafwala	Pakistan
36	15024	PARC/IBPGR	Pakistan	73	sahiwal-2002	MMRI,Yousafwala	Pakistan
37	15026	PARC/IBPGR	Pakistan				

Protein extraction: Two seeds from each genotype were crushed to fine powder. 0.05 gram of fine flour were put into 1.5ml micro-centrifuge tube. 950 µl of protein extraction buffer

(62.5mM tris HCl (pH 6.8)), 2.3% SDS, 5% 2-ME, 10% glycerol, 0.1% bromophenol blue) was added to each micro-centrifuge tube and vortexed for 5minutes to mix well. kept the mixture at room temperature for 1hour and centrifuged at 3000Xg for 5minutes. After centrifugation, the crude proteins in the form of supernatant were transferred carefully to fresh micro-centrifuge tube and kept at -20ºC till electrophoretic separation.

Preparation of electrophoretic gel: To identify genetic diveristy by using SDS-PAGE, the extracted crude proteins were profiled through 12% (w/v) concentrated discontinuous polyacrylamide gel using Laemmli (1970) protocole, while that of stacking gel was 4.5% (w/v) (Walter et al., 2003). The two vertical glass plates were fixed togather marked two centimeters from the top. The separating gel comprised 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 15µl tetramethylene-diamine (TEMED) and 200 µl ammonium per sulphate (APS). The stacking gel consisted of 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 17µl Tetramethylene-diamine (TEMED) and 70 µl ammonium per sulphate (APS). The electrode buffer composed of Tris-glycine (43.2gm glycine and 9gm Tris-HCl per 3liters buffer solution at pH 8.9) with 3gm SDS(0.1%). Eight microliters of the seed sample was loaded into the each well along with protein ladder of known molecular weight (Fermentas Life Sciences) lied to the extreme left of the stacking gel.

Electrophoresis: Electrophoresis was carried out at 100V for 3hours till Bromophenol blue marker reached bottom of the gel. After electrophoresis the gels were stained in staining solution (methanol 44%, acetic acid 6%, commassie breliant blue .225%) for one hour and then transferred to destaining solution

(methanol 20%, acetic acid 5%) and kept on shaker over night.

Data Analysis: Based on the absence or presence of protein bands, similarity index was planned for all potential pairs of polyoeotide types. The intensity of protein sub-units were not considered to avoid taxonomic weighing but only interpreted them in terms of presence or absence as 0 for absence and 1 for the presence of protein bands. The data was compiled and subjected to binary data matrix. On the basis of outcome of electrophoretic band specra, the Nei and Li's similarity matrix was calculated for all conceivable sets of protein type's electrophoregrams by means of the following formula (Sneath and Sokal, 1973):

$$S = W/(A+B-W)$$

Results

A total of 18 scorable protein bands were detected as a result of SDS-PAGE technique. On the basis of these protein subunits 73 genotypes of maize (Table 1) were evaluated. Out of these, 39 percent (7 protein subunits) were consistanty present throughout the genotypes and considered as monomorphic while 61percent (11 protein subunits) replected variations and designated as polymorphic. The size of the polypeptide bands varied between 10 and 122 kDa. A pre-stained protein ladder of known molecular weight ranged between 10 and 170 kDa (Fermentas Life Sciences) was used as marker for the calculation of the molecular weight of protein subunits. The conserved polypeptide sequences were 2,3,8,9,11,12 and 15. The polypeptide sequence number 13 with molecular weight of was present only in 14911, 15117, 15215, 15227, 15258, 15262 and Sahiwal-2002 while the polypeptide sequence number 17 was absent in only 14880, 14914, 15162 and 15244.

Gel was demarcated into four zones based on

polypeptide banding profiles i.e. A, B, C and D (Fig. 1, Table 1). The major protein subunits were present in zone A and C while minor in zones C and D. It was deteced that different genotypes showed more variability in seed proteins in minor polypeptide bands as compared to major protein subunits. Zone A (>51 kDa, MW) comprised of 8 protein subunits in which three were monomorphic and five polymorphic, zone B (28-51 kDa, MW) consisted of 2 protein subunits in which one was monomorphic and one polymorphic, in zone C (17-27 kDa, MW) out of 4 protein subunits, 2 were monomorphic and 2 polymorphic and zone D (10-16 kDa, MW) had a total of 4 protein bands in which one was monomorphic and three polymorphic. In the light of these facts zone A and Dwere having more polymorphism and potentialy more suitable ranges for the assessment of genetic diversity of maize germplasm.

To construct dendogram, the coefficient of similarity matrix of 73 genotypes as a result of SDS-PAGE, through UPGMA method was used, by utelizing NTSYS-PC, vesion 2.1. Dendogram separated the whole tested genotypes into two main groups, I and II at 84 percent homology (Fig.3). Cluster 'I' was the smallest group with 7 genotypes i.e. 14911, 15227, 15258, Sahiwal-2002, 15117, 15262 and 15215 while group 'II' the largest group with 66 genotypes. Group 'II' was further subdivided into two clusters, A and B. Cluster 'A' was the largest one with 63 genotypes and cluster 'B' was the smallest with only 3 accessions. Cluster 'A' was further divided into sub-cluster 'A1' with 38 genotypes and sub-luster 'A2' with 25 genotypes, the largest included Agaiti-2002.. Sub-cluster 'A1' was further consisted of two smaller groups 'A1a' with 19 accessions and ' A1b' also with 19 genotypes including Sadaf. Similarly sub-cluster 'A2' was further comprised of smaller groups 'A2a' with 15 genotypes including Agaiti-2002 and 'A2b' with 10 accessions. During the present invistigation the separation of the whole genotypes into two main groups and the convergence of 66 genotypes into one proup i.e. group 'II'

replected that low level of genetic veriability is present in the tested maize germplasm. This degree of heterogeneity may be atributed to their narrow genetic background.

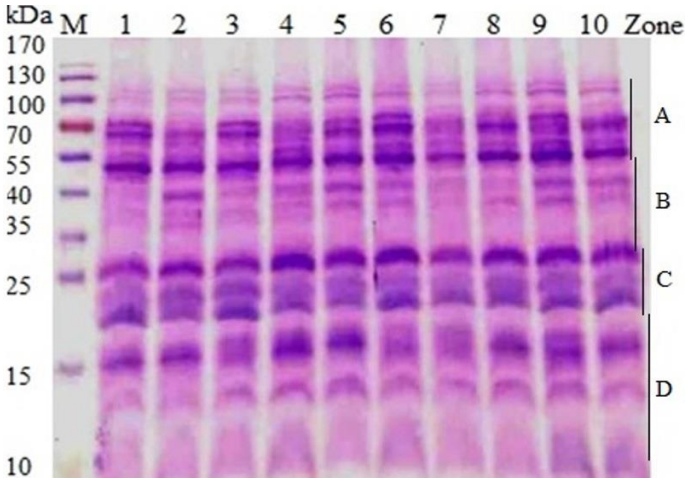

Figure 1: Protein profile showing total seed storage proteins in maize genotypes as a result of SDS-PAGE.
M= Protein Ladder, 1=15172, 2=15209, 3=15232, 4=15258, 5=15262, 6=15263, 7=15264, 8=15265, 9=15277, 10=15278.

Table. 1: Summary of the total seed storage protein polypeptides of maize in the gel

Zone	Range (MW in kDa)	No of Bands	Polymorphic Bands	Monomorphic Bands	Percent Polymorphism	Contribution to the total polymorphism
A	52- 22	8	5	3	62.5	27.8
B	28-51	2	1	1	50	5.5
C	17-27	4	2	2	50	11.1
D	10-16	4	3	1	75	16.7
Total	10-122	18	11	7		61.1

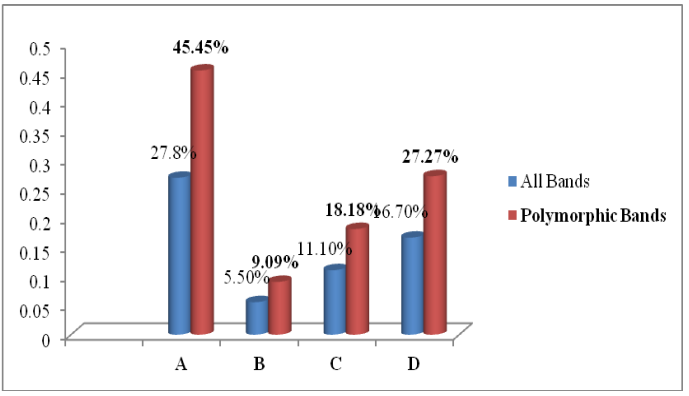

Figure 2: Percentage contribution of different zones among all bands and polymorphic bands.

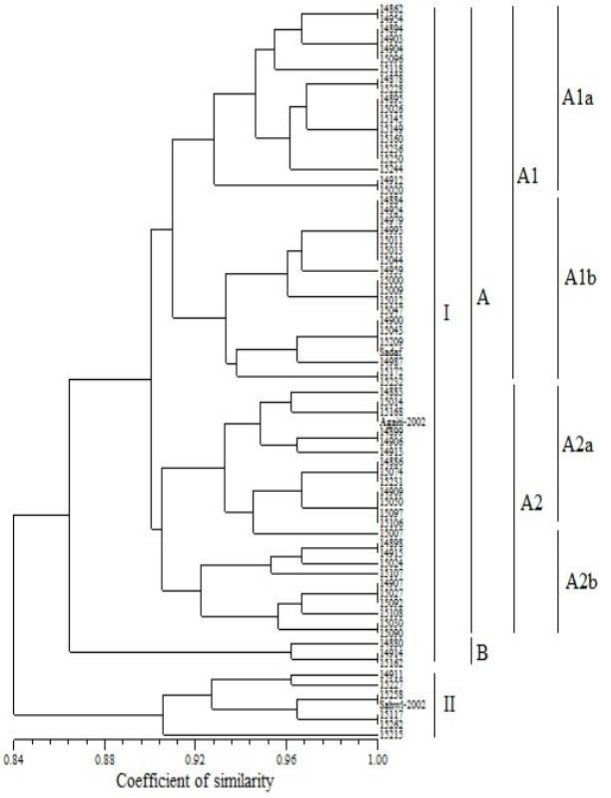

Figure 3: Dendrogram showing the relationship among maize genotypes in the light of total seed storage protein study through SDS-PAGE.

Discussion

To conserve the genetic diversity of crops is one of the mile stones to be achieved especially due to post green revolution consequences. It is obvious that the elucidation of genetic diversity is extremely necessary for the effective maintenance, evaluation and utilization of germplasm because germplasm is the only source to be exploited for the development of new varieties during breeding programs (Baranger, 2004). Proteins have been used as markers to estimate the genetic diversity in many crops species (Nagy *et al*., 2009). These are the decoded informations of their DNA (Motto *et al*., 1989) which can be used to characterize the genetic diversity in maize (Nucca *et al.,*1978). Total seed storage proteins replect high level of polymorphism, their function is the product of gene and their electrophoretic banding pattern is very minutly affected by environment (Gepts *et al*., 1986). Seed storage protein banding pattern is utilized for the identification of varieties, characterization of plant germplasm, studying phylogeny of different species and analysis in biosystematics (Sammour, 1991). Seed storage proteins are extensively explored in maize worldwide, mostly by applying the SDS-PAGE technique (Koranyi 1989; Poperelya *et al.,*1989; Wang *et al*., 1994; Gorinstein *et al*., 1999; Shah *et al*.,2003; Abdel-Tawab 2004 and Anjali and Sanjay 2012). The SDS-PAGE technique is cheap and easy method to estimate germplasm diversity.

In the present study an attempt has been made to give a blue print of the genetic diversity assessment of 73 genotypes of maize including both indigenous and exotic germplasm through SDS-PAGE technique. A total of 18 scorable protein subunits were recorded. Out of these, 39 percent (7 protein subunits) were monomorphic and 61 percent (11 protein subunits) polymorphic which are in line with the findings of (Rashed *et al*., 2010) who reported a total of 16 protein subunits, out of which 38 percent (6 protein subunits) were

monomorphic and 62 percent (10 protein subunits) polymorphic. The protein banding pattern replected variability in the gel pictures which agrees with the method followed by Shah (1999). Major as well as minor protein subunits showed variation which is congruent with the results of (Gorinstein *et al*., 2004), who detected veriation in major protein subunits, minor protein subunits and fine structure as well. Genetic variability estimation was carriedout on the basis of presence and absence of polypeptide bands. It was also noted that non of the genotypes possess all the 18 protein subunits collectively, which rsulted in the destribution of all 73 genotypes into distinct protein banding patterns. Our results are concordant with findings of Ladizinsky and Hymowitz (1979), who stated that seed protein electrophoresis has become a powerful tool by the protein profile stability and additiveness to explore the origin and evolution of crops.This digree of veriabilty is less in major protein subunits than the minor polypeptides.

Among the major polypeptide chains the protein subunit with molecular weight of 21kDa was present in only 7 genotypes (14911, 15117, 15215,15227, 15258,15262, Sahiwal-2002). It replected that the gene coding this protein subunit is only present in these genotypes. Another major polypeptide chain with 19kDa was absent in only 8 genotypes but present in rest of the germplasm which showed the presence of gene for this protein in rest of the germplasm. These results were in agreement with the findings of Shah *et al* (2003), who reported that genetic diversity is present in maize genotypes due to the presence or absence of gene(s). Among the minor protein subunits 69.23 percent (9 protein subunits out of 13) showed genetic veiability among the tested genotypes. There was no significant correlation between the variability detected during total protein study and the geographical distribution of the studied maize germplasm because the accessions collected from the same locality, distrbuted into separate clusters. These results are supported by the findings of Sanni *et al*. (2008). The

slight variation was noted in the present investigation in comparison with the findings already reported, which may be due to the variability in the genetic background of the material used and percentage and size of the gel used to separate the protein sub-units.

It is concluded from the present study that more diverged intra specific genotypes could be studied by using the SDS-PAGE technique. It seems to be effective tool for the maintenance, evaluation and utilization of maize germplasm which is the only source to be utilized for the development of new varieties during breeding programs.

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