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INVESTIGATION OF TOTAL SEED STORAGE PROTEINS OF PAKISTANI AND JAPANESE MAIZE (*ZEA MAYS* L.) THROUGH SDS-PAGE MARKERS

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Abstract

The assessment of genetic diversity among the members of a species is of vital importance for successful breeding and adaptability. In the present study 83 genotypes of maize of Pakistani and Japanese origin were evaluated for the total seed storage proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) through vertical slab unit. The total protein subunits were separated on 12% polyacrylamide gel using standard protocols. A total of 18 protein subunits were noted out of which 7 (39%) were monomorphic and 11 (61%) were polymorphic, with molecular weight ranging from 10 to 122 kDa. Coefficients of similarity among the accessions ranged between 0.89 and 1.00. The dendrogram obtained through UPGMA clustering method showed two main clusters: 1 and 2. First cluster comprised of 9 genotypes including Sahiwal-2002, while second cluster contained 74 genotypes including Aaiti-2002 and Sadaf. Over all a low level of polymorphism was observed in total seed storage protein patterns of maize genotypes from Pakistan as well as Japan. It is inferred from the present study that more genotypes of maize could be brought under study and more advanced biochemical techniques with more reliable results could be followed to bring assessment of genetic diversity of maize for planning breeding programs.

Introduction

Maize (*Zea mays* L.) is an annual, cross-pollinated by wind and the only monoecious among cereal crops to have male and female inflorescences on separate branches of the same plant. It belongs to grass family Poaceae (Gramineae) which is leading in importance in the order Poales (Bremer *et al*., 2003). This family contributes to the world economy, food and industry through valuable crops i.e. wheat, rice and maize (Mabberley, 2008). Being most domesticated with controversy in origin and evolution, there is one school of thoughts that maize is the nearest descendant of Mexican teosinte (Dowswell *et al.,* 1996). There is no doubt that human beings directly or indirectly depend on plants for various purposes for which they domesticated these with the passage of time and flourished with spreading communities, undergone through evolution, passing through various cultivating methodologies throughout the world (Larik, 1994).

In Pakistan maize is third in importance after wheat and rice (Chaudhry, 1994) with average yield of 3415 kg ha^{-1}, while second in importance after wheat in Khyber Pakhtunkhwa (Asif *et al.,* 2007) with average yield of 1880 kg ha^{-1} (Anon., 2009). It is potentially high yielding and needs less effort as compared to other cereal crops (Abdullah, 1999). It is one of the important sources of proteins throughout the world i.e. gives almost 42 million tons proteins per year which is about 15% of the proteins obtained from food-crops annually throughout the world (Li & Vassal, 2004).

Maize is one of the most diverse crop species with great potential to grow in a wide range of environments, from equatorial region to high latitudes in north and south, from sea level to over 3000 meters. It is the genetic plasticity of a species that makes it able to bear different kind of stresses and adjust itself in new challenging environment. Such degree of genetic variability in morphological and physiological traits is not possible in other crop species (Mangelsdorf, 1974). The value of broad genetic makeup is quite understandable because narrow genetic makeup always faces the threat of genetic vulnerability to different stresses (Masood *et al*., 2005). That is why the assessment of genetic diversity of maize germplasm is very fruitful for planning breeding programs and conserving germplasm.

There are different methodologies to investigate the genetic diversity in maize. Usually assessment is done through agro-morphological traits which are effective but do not give consistent results because of the possibility of affecting by different environmental factors. Some time gives such type of results that are not the interpretation of their genetic background and shows a kind of flaw to solely use as a tool in germplasm characterization. It is also expensive and time consuming. To overcome these short comings, it is needed to look for more effective and reliable technique(s). One of these is biochemical study particularly proteins (Wallace *et al*., 1990) which is based on the separation of protein into specific banding pattern. The cereal grain proteins study is traced back for more than 268 years when wheat gluten was studied for the first time in 1745 (Beccari, 1745). Later on Osborne (19f07) who is considered as father of plant protein chemistry and many others made their efforts in this regard.

Protein markers copy the information stored in DNA which can be a good source for effective estimating of genetic plasticity in maize germplasm. In protein fingerprinting the extraction of proteins from different parts of plant i.e. leaves, shoots, seeds etc and their electrophoresis is done. Polyacrylamide gel electrophoresis played major part and made possible to know about variation in the physical and chemical characteristics of proteins (Akbar *et al.*, 2012). To identify and characterize various cultivars the electrophoresis of seed protein is effective technique as

it is reproducible, rapid and cost effective (Laemmli, 1970). Variations between hybrids and lines can be carried out through sodium dodecyl sulphate polyacrylamide gel electrophoresis (Koranyi, 1989). Through electrophoresis the hybrids of F_1 generation and degree of hybridity in a seed lot can be studied (Poperelya *et al*., 1989). The assessment of maize genetic purity and variety identification can be done in effective way through sodium dodecyl sulphate polyacrylamide gel electrophoresis (Khan *et al*., 2013). Protein bands in the polyacrylamide gel translate their genetic background through which the wild or newly made cereals from wild parents can be studied (Gorinstein *et al*., 1999). For studying the degree of genetic variability in maize seed storage proteins, the sodium dodecyl sulphate polyacrylamide gel electrophoresis has been fruitful (Paulis *et al*., 1975). It has been investigated that along with physiochemical and molecular study, SDS-PAGE is impressive way to elucidate the genetic variability in seed storage protein (Zeb *et al*., 2006). The electrophoretic separation of proteins results in the characteristic banding pattern which has been used with good results to estimate the genetic diversity in different crops like wheat, rice, cotton, etc. During the present experimentation 83 accessions of maize were investigated to assess the variability and differentiation in total seed storage proteins. The objectives were to carry out the assessment of genetic variation among maize accessions through the electrophoretic separation of proteins with different molecular weights through SDS- PAGE.

Materials and Methods

Plant material: Plant material consisted of 83 accessions of maize (Table 1) that were obtained from the Gene-bank of Plant Genetic Resources Institute, National Agricultural Research Centre, Islamabad.

Protein extraction: Whole seeds were crushed to fine powder and 0.05 gram of fine flour were put into 1.5ml 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 flour in the centrifuge tube and vortexed for 5 minutes to mix well. Then kept at room temperature for 1 hour and cerifuged for 5 minutes at 3000Xg. After centrifugation trasnfered the supernatent to fresh centrifuge tube and kept at -4ºC till electrophoretic separation.

Table 1. List of maize accessions used in present investigation.

No.	Accession	Origin	No.	Accession	Origin	No.	Accession	Origin
01.	15263	Japan	29.	15333	Pakistan	57.	24677	Pakistan
02.	15264	Japan	30.	15334	Pakistan	58.	24678	Pakistan
03.	15265	Japan	31.	15336	Pakistan	59.	24679	Pakistan
04.	15276	Japan	32.	15338	Pakistan	60.	24680	Pakistan
05.	15277	Japan	33.	15339	Pakistan	61.	24682	Pakistan
06.	15278	Japan	34.	15340	Pakistan	62.	24683	Pakistan
07.	15279	Japan	35.	15341	Pakistan	63.	24684	Pakistan
08.	15280	Japan	36.	15342	Pakistan	64.	24685	Pakistan
09.	15290	Japan	37.	15343	Pakistan	65.	24686	Pakistan
10.	15296	Japan	38.	15345	Pakistan	66.	24687	Pakistan
11.	15302	Japan	39.	15346	Pakistan	67.	24688	Pakistan
12.	15304	Japan	40.	15347	Pakistan	68.	24689	Pakistan
13.	15306	Japan	41.	15348	Pakistan	69.	24690	Pakistan
14.	15308	Japan	42.	15349	Pakistan	70.	24691	Pakistan
15.	15310	Japan	43.	15350	Pakistan	71.	24692	Pakistan
16.	15311	Japan	44.	15351	Pakistan	72.	24693	Pakistan
17.	15317	Pakistan	45.	15352	Pakistan	73.	24694	Pakistan
18.	15318	Pakistan	46.	15353	Pakistan	74.	24695	Pakistan
19.	15319	Pakistan	47.	19188	Pakistan	75.	24696	Pakistan
20.	15321	Pakistan	48.	19195	Pakistan	76.	24697	Pakistan
21.	15325	Pakistan	49.	19198	Pakistan	77.	24698	Pakistan
22.	15326	Pakistan	50.	24669	Pakistan	78.	24699	Pakistan
23.	15327	Pakistan	51.	24670	Pakistan	79.	24700	Pakistan
24.	15328	Pakistan	52.	24671	Pakistan	80.	24701	Pakistan
25.	15329	Pakistan	53.	24672	Pakistan	81.	Agaiti-2002	Pakistan
26.	15330	Pakistan	54.	24674	Pakistan	82.	Sadaf	Pakistan
27.	15331	Pakistan	55.	24675	Pakistan	83.	Sahiwal-2002	Pakistan
28.	15332	Pakistan	56.	24676	Pakistan			

Preparation of electrophoretic gel: SDS-PAGE of total seed storage proteins was carried out in 12% polyacrylamide gel using Laemmli (1970) protocols. 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 15µl tetramethylene-diamine (TEMED) and 200µl ammonium per sulphate (APS). The stacking 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 17µl tetramethylene-diamine (TEMED) and 70µl ammonium per sulphate (APS). The electrode buffer contained Tris-glycine (43.2g glycine and 9g Tris-HCl per 3 liters buffer solution at pH 8.9) with 3g SDS (0.1%). Eight microliters of sample was loaded into the each well of the stacking gel.

Elecrophoresis: Electrophoresis was carried out at 100V for 3 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 stansards (Fermentas Life Sciences). After electrophoresis the gels were stained in staining solution (methanol 44%, acetic acid 6%, commassie breliant blue 0.225%) 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 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):

$$\text{Similarity (S)} = w/(a+b-w)$$

where w = number of bands of common mobility; a = number of bands in protein type 'a' and b = number of bands in protein type 'b'.

Results

The number of total scorable protein bands was eighteen as a result of SDS-PAGE technique but those that were not cosistent in reproducibility and showed occasional variation in sharpness and density were not considered. Based on these bands eighty three accessions of maize (Table 1) were screened. Out of eighteen polypeptide bands, 7 (39%) were commonly present in all accessions and considered as monomorphic, while 11 (61%) showed variations and considered as polymorphic. The size of the protein bands obtained through SDS-PAGE ranged from 10 to 122 kDa. A pre-stained protein marker with molecular weight ranging from 10 to 170 kDa (Fermentas Life Sciences) was used for reference to calculate the molecular weight of the polypeptide bands. The bands that were present in all of the maize lines were band number 2, 3, 8, 9, 11, 12 and 15. Band number 13 was present only in 15263, 15265, 15332, 24670, 24683, 24690, 24695, 24699 and Sahiwal-2002, while band number 17 was absent in only 15311, 15321 and 24677.

On the basis of banding profiles of proteins of different kDa, gel was divided into zones A, B, C and D (Figs. 1 & 2; Table 2). The major protein bands were lied in zones A and C, while minor bands were present in zones C and D. It was noted that different accessions of maize showed more diversity in seed storage proteins in minor bands in comparison to major bands. In zone A (MW>51 kDa) out of 8 protein bands, 3 were monomorphic and 5 were polymorphic. In zone B (28-51 kDa, MW) out of 2 protein bands,1 was monomorphic and 1 was polymorphic. In zone C (17-27 kDa, MW) out of 4 protein bands, 2 were monomorphic whereas 2 polymorphic. And in zone D (10-16 kDa; MW) out of 4 protein bands, 1 was monomorphic and 3 were polymorphic. By considering these facts zone A and D were more polymorphic and could be more suitable areas for assessment of genetic diversity of maize germplasm.

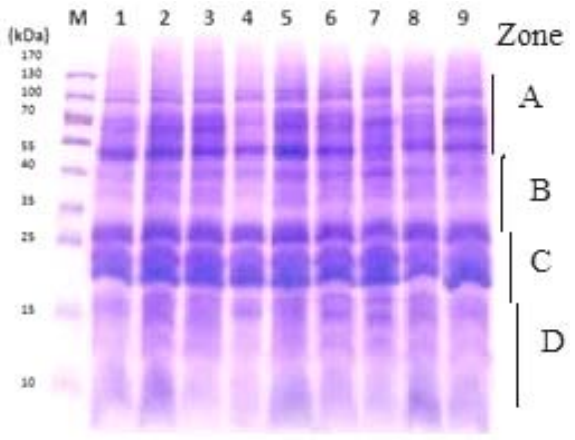

Fig. 1. Maize genotpes showing protein bands as a result of SDS-PAGE. M = Protein ladder, 1 = 15340, 2 = 19195, 3 = 19198, 4 = 24674, 5 = 24678, 6 = 24680, 7 = 24682, 8 = 24683, 9 = 24684.

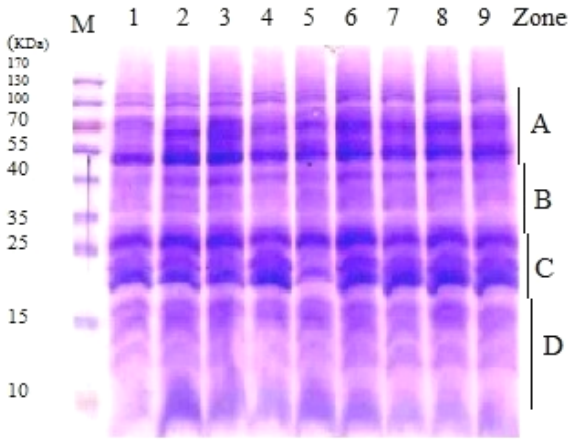

Fig. 2. Maize genotpes showing protein bands as a result of SDS-PAGE. M = Protein ladder, 1 = 24685, 2 = 24686, 3 = 24688, 4 = 24689, 5 = 24690, 6 = 24691, 7 = 24692, 8 = 24693, 9 = 24694.

Table 2. Summary of the total seed storage protein bands of maize in the gel.

Zone	MW Range (kDa)	No. of bands	Polymorphic bands	Monomorphic bands	Percent polymorphism
A	52-122	8	5	3	62.5
B	28-51	2	1	1	50
C	17-27	4	2	2	50
D	10-16	4	3	1	75
Total	**10-122**	**18**	**11**	**7**	**61.1**

For the construction of dendogram, the coefficient of similarity matrix of 83 genotypes as a result of SDS-PAGE, through UPGMA method was used, utilizing NTSYS-PC, vesion 2.1. Dendogram showed two main clusters 1 and 2 at 89% homology (Fig. 3). Cluster □1□ is the smaller group with nine genotypes i.e. 15264, 15276, 15332, 24670, 24683, 24690, 24695, 24699, and Sahiwal-2002, while cluster □2□ the larger group with 74 genotypes. Cluster □2□ was further sub-divided into two sub-clusters, I and II. Sub-cluster I was the larger one with 41 genotypes and sub-cluster II was the smaller with 22 genotypes. Sub-cluster □I□ was further divided into sub-group A_1 with 12 lines, the smallest one included Sadafand B_1 with 29 lines, the largest included Agaiti-2002. Sub-cluster □II□ was further sub-divided into sub-group A_2 with 15 accessions and sub-group B_2 with 7 accessions. During the present invistigation the division of whole genotypes into only two sub-clusters and the convergence of 74 accessions into one proup i.e. II, reflected low level of genetic variability. This low degree of heterogeneity may be due to their narrow genetic background. Similarly the present study of genetic variability in the seed storage polypeptide determined by SDS-PAGE technique proved that it is fruitful to identify genetic diversity among accessions of maize.

Discussion

Protein markers have the decoded instructions of their genetic background i.e. DNA (Motto *et al*., 1989) which can be used to estimate genetic variability in maize (Nucca *et al.*,1978). These have been used to discriminate the germplasm diversity in many crops (Nagy *et al*., 2009). Total seed proteins showed high level of polymorphism, their function is the product of gene and there is very little effect of the environment on their electrophoretic banding pattern (Gepts *et al*., 1986). Seed torage proteins became an efficient tool to study genetic plasticity and have been successfully adapted to identify varieties in many crop species. There is extensive study of seed storage proteins in maize through out the world in which mostly the SDS-PAGE technique is followed (Koranyi 1989; Poperelya *et al*., 1989; Wang *et al*., 1994; Gorinstein *et al*., 1999; Shah *et al*., 2003; Abdel-Tawab 2004; Anjali & Sanjay, 2012). The SDS-PAGE technique is cheap and easy method to estimate germplasm variability.

In the present study it has been attempted to give a blue print of the whole experiment in the context of genetic diversity assessment of 83 genotypes of maize including both indigenous and exotic germplasm through SDS-PAGE technique. A total of 18 scorable protein bands were considered, out of which 7 (39%) were monomorphic and 11 (61%) were polymorphic. Results agreed with the findings of Rashed *et al*., (2010) who found a total of 16 protein bands, out of which 6 (38%) were monomorphic and 10 (62%) polymorphic. The variation was noted both in major and minor protein bands which is supported by the results of Gorinstein *et al*., (2004) who noted that variation was found in major bands, minor bands and in fine structure as well. Genetic diversity assessment was done on the basis of presence and absence of protein bands and found that there was no such genotype to possess all the 18 protein bands collectively which resulted in the identification of all 83 genotypes into distinct protein banding patterns. This is supported by the results of Ladizinsky & Hymowitz (1979) who stated that seed protein electrophoresis has made a powerful tool by the protein profile stability and additiveness to study the origin and evolution of crops. This degree of variation is less in major protein bands as compared to minor bands.

Only in 2 out of 5 major protein bands, the variability was found in a few genotypes such as protein subunit of molecular weight (MW) 21kDa (band 13) was shared by only 9 genotypes (15264, 15276, 15332, 24670, 24683, 24690, 24695, 24699, Sahiwal-2002) out of 83 genotypes which means that these genotypes are similar in having gene for coding this protein subunit. Similarly 19kDa (band 14) was present in 74 genotypes but absent in 9 genotypes which means that all these having gene for this protein which is supported by the findings of Shah *et al*., (2003) who stated that maize genotypes having similarities or differences because of the presence or absence of gene(s). While in 8 out of 13 minor peptide bands the variation was noted among many genotypes.

It is infered from the present investigation that through seed storage proteins electrophoresis, the identification of more diverse genotypes within a species could be possible which is not only important for quality control and maintaining purity but also enhancing the opportunities for effective breeding program within no time, less effort and less money wasted. The low degree of variation was found in maize which is needed to be studied by using other biochemical/molecular markers.

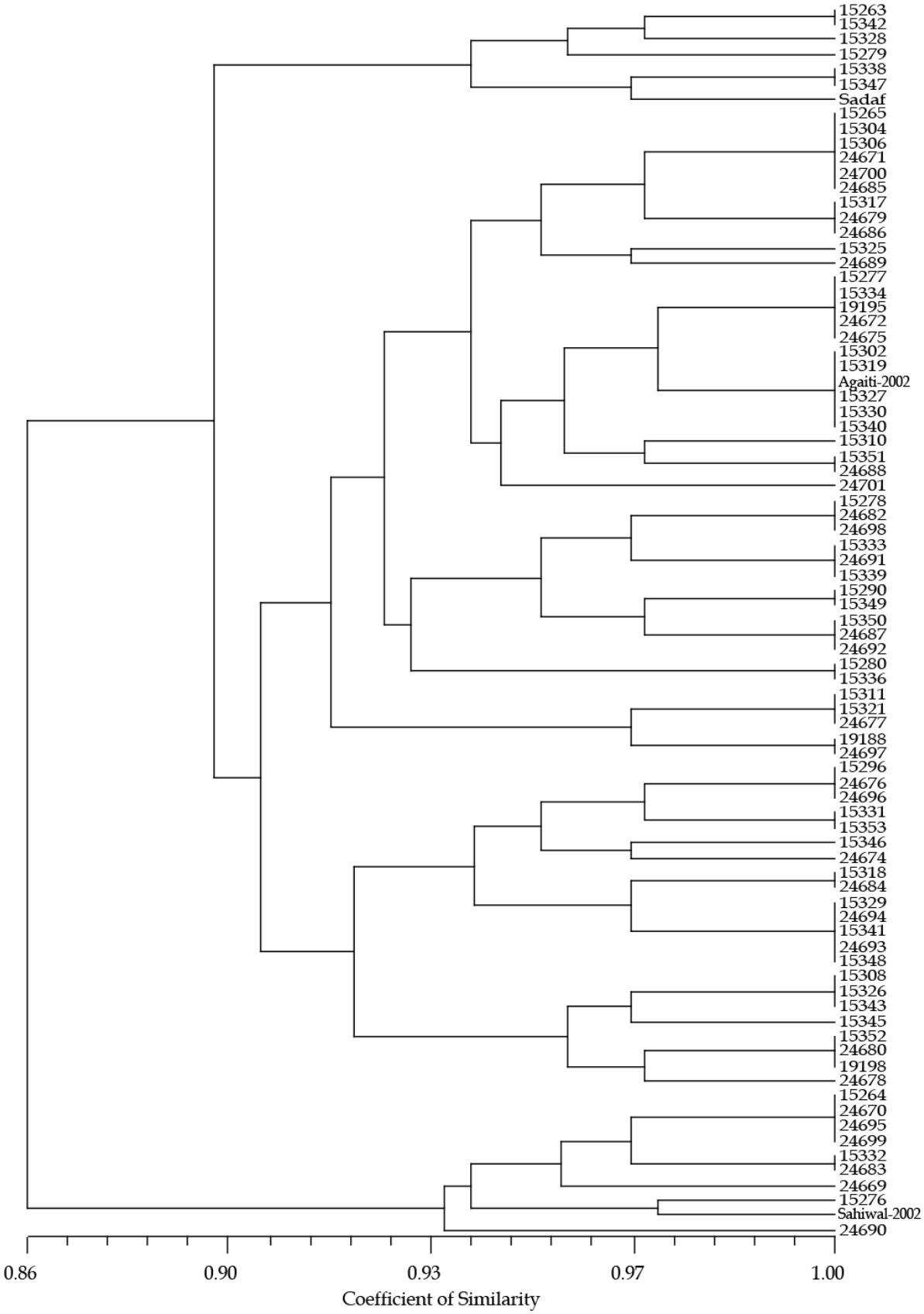

Fig. 3. Dendrogram showing the relationship among maize genotypes in the light of total seed storage protein study through SDS-PAGE.

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