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Genetic diversity of maize (*Zea mays*) hybrids based on protein analysis in different conditions

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ABSTRACT

Genetic variability of maize (*Zea mays* L.) hybrids grown in the fields is very important because genetic uniformity implies risk of genetic vulnerability to stress factors and cause great loss in yield. Thus, knowledge of maize germplasm genetic diversity is important for planning breeding programmes, germplasm conservation per se. The systematic study to know the genetic architecture of the cultivars and their progenies has been improved by the incorporation of biochemical analysis especially of proteins as they portray the genetic base with more fidelity. Generally seed storage proteins have been used as markers in the analysis of genetic diversity within and among population as they are an impact of the genetic information existing on DNA. Under the present study the genetic diversity of 21 maize cultivars based on embryo protein markers through SDS & Native PAGE protein analysis was revealed differentiation from each other in both conditions. In SDS-PAGE more number of bands (22) was observed as compared to native PAGE (17). The dendogram using UPGMA was constructed on basis of similarity matrix calculated by the presence/absence of protein fractions. Further clustering of maize accessions in dendogram were more in accordance with morphological traits like plant height, cob diameter etc. as compared to native PAGE protein profiling. Thus, it can be concluded that SDS-PAGE either separately or together with agronomic traits is useful for genetic diversity studies in maize.

Key words: Dendogram, Protein profiling, Similarity matrix, UPGMA

Maize (*Zea mays* L.) is one of the most diverse crop species containing tremendous variation in morphological and physiological traits which allows it to be cultivated in a range of environments. This diversity of maize inbreeds has major importance in the process of improvement. Genetic variability of maize hybrids grown in the fields is also very important because genetic uniformity implies risk of genetic vulnerability to stress factors and cause great loss in yield. Thus, knowledge of maize germplasm genetic diversity is important for planning breeding programmes, germplasm conservation per se. (Bauer *et al*. 2005)

Different methodologies are available to investigate genetic diversity. Analysis based on morphological and agronomic traits along with pedigree data have been used for this purpose for long time. Morphological characteristics are often influenced by environment and do not always express genetic relationships. They are also expensive and time consuming. Thus, the systematic study to know the genetic architecture of the cultivars and their progenies has been improved by the incorporation of biochemical analysis especially of proteins as they portray the genetic base with more fidelity (Murtaza *et al*. 2005). Generally seed storage proteins have been used as markers in the following four main areas: analysis of genetic diversity within and among population, plant domestication in relation to genetic resources conservation and breeding, genome relationships especially in polyploid series and as a tool in plant breeding (Asante *et al*. 2008). Protein markers are an impact of the genetic information existing on DNA and thus, can be used for efficient determination of genetic diversity (Nagy *et al*. 2009)

The objective of the present study was to determine the genetic diversity of 21 maize cultivars based on embryo protein markers through SDS & Native PAGE protein analysis. Comparison of the results from these markers in also done with morphological and their pedigree information.

MATERIALS AND METHODS

The seeds of 21 maize cultivars used in (Table 1) this study for protein extraction were collected from Department of Genetics and Plant Breeding, G B Pant University of Agriculture & Technology.

For SDS-PAGE seed power (20 mg) was mixed in 150µl SDS-PAGE sample buffer (pH 6.8) containing 0.125M Tris Cl, 4% SDS, 3% 2-mercaptoethanol, 20% glycerol and 0.02%

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Table 1 Growth, yields attributes and yields of maize (mean of 2 years)

Accessions	Plant height (cm)	Cob length (cm)	No. of grain rows/cob	1000-seed wt (g)	Grain yield (t/ha)
Pant 97K 1038	257	32.0	16.33	29.67	6.51
Pant 97K 1040	247	32.0	15.34	37.67	6.59
Pant 97K 1041	280	29.6	16.00	36.67	5.17
Pant 97K 1042	270	30.8	17.67	35.09	4.99
Pant 97K 1043	225	25.6	16.63	29.67	5.17
Pant 97K 1044	228	28.5	15.24	30.50	6.99
Pant 97K 1045	209	29.3	14.30	31.50	4.29
Pant 97K 1046	210	27.3	13.27	32.30	4.62
Hyd 97 R 111	286	32.3	16.50	39.90	6.72
Hyd 97 R 112	295	33.3	15.40	36.30	6.92
Hyd 97 R 113	293	31.3	15.90	35.30	6.93
Hyd 97 R 116	268	28.9	16.00	36.70	6.77
Pant 97K (1083X1082)	256	28.5	14.90	36.80	5.51
Pant 97K (1087X1086)	249	26.5	15.3	33.30	5.59
Pant 97K (1123X1124)	252	28.9	16.10	34.30	5.69
Pant 97K (1091X1090)	235	28.5	13.20	31.30	4.39
Pant 97K (1097X1096)	281	26.5	14.30	32.50	6.77
Pant 97K (1109X1108)	221	26.5	14.90	33.30	5.69
Pant 97K (1115X1114)	236	27.7	14.90	35.20	5.63
Pant 97K (1120X1121)	253	26.9	15.30	35.90	6.43
Pant 98K (1010X1011)	241	25.3	15.90	38.30	6.31
Pant 98K (1014X1015)	248	30.1	16.30	35.89	6.30

bromophenol blue. It was then heated for 5 minute in hot water bath and then centrifuged at 10000 rpm for 10 min. the supernatant where then loaded on to the gel. Protein samples were electrophosed in a discontinuous SDS-PAGE gel following Leammli (1970) using a 15% separating gel (0.375M Tris Cl; pH 8.8) and 5% stacking gel (0.125 m Tris Cl; pH 6.8) in tris glycine buffer (0.025M Tris; pH 8.3, 0.192 M glycine; 0.1% SDS). Electrophoresis was carried out at 20m A current (5-6 hr) until the proteins move to the bottom of the gel. Staining of the gel was done using 0.2% (w/v) Coommassie brilliant Blue R- 250 in 12.5% (w/v) trichloroacetic acid. The position of the protein bands in the gel was expressed to compare with standard protein markers of known molecular weight.

For PAGE analysis ground seed powder (10 mg) was taken and mixed with protein extraction solution in plastic centrifuge tube, containing 0.05m sodium chloride with 20% (w/v) sucrose and 0.4% (w/v) bromophenol blue, added in 1:4 (w/v) ratio. Samples were mixed thoroughly and centrifuged for 15 minutes at 10000 rpm. The supernatant were applied to the gel. A high resolution PAGE under acidic condition were performed according to the method of Wang Chun *et al.* (1994) with some modifications. The 15% continuous gel was prepared by mixing acrylamide (18.6% T, 3.3% C), 0.1 M sodium formate (pH 3.2), water, TEMED, APS (0.5% w/v) in electrode buffer (pH 3.45 of glycine formic acid). The electorphoresis was carried out at 180 V constant voltage in the reverse polarity at 4^0 C for 7-8 hr. After electrophoresis staining and distaining of the gel was done as in SDS PAGE.

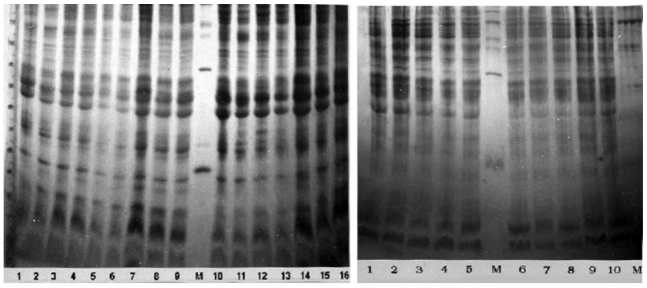
Presence/absence of protein fractions was transformed to binary data and co-efficient of similarity according to Jaccard (1908) was calculated and dendogram was constructed using UPGMA method.

RESULTS AND DISCUSSION

The analysis of embryo salt soluble protein in SDS-PAGE, i e under denaturing condition showed 22 numbers of bands with their relative mobility ranging from 02 to 0.89, with molecular weight in range of 100 to 10 Kda (Fig 1). Presence/absence of protein fractions were transformed to binary data and co-efficient of similarity (Jaccard 1908) was calculated (Table 2).

The analysis of embryo salt soluble proteins in non-denaturing condition, i e PAGE showed 17 numbers of bands with their relative mobility in the range of 0.05 to 0.89 (Fig 3). The dendogram (Table 3) was constructed on basis of similarity matrix calculated by the presence/absence of protein fractions.

Lowest similarity (2.6%) has been observed between Pant 97K 1097 X 1096 and Hyd 97R 113 as revealed by SDS-PAGE analysis of total soluble protein (Table 2), whereas native PAGE revealed these two were only 11.1% similar (Table 3). Based on PAGE similarity matrix lowest similarity was 0% amongst number of accessions while it was not observed in SDS-PAGE. In SDS-PAGE highest similarity of



Pant 97K 1038
Pant 97K 1040
Pant 97K1045
Pant 97K1046
Pant 97K1044
Pant 97K1083 X 1082
Pant 97K1123 X 1124
Pant 97K1115 X 1114
Pant 97K1120 X 1121
M Molecular weight marker
Pant 97K1041
Pant 97K1042
Pant 97K1043
Pant 97K1087 X 1086
Pant 97K1091 X 1090

Hyd 97R 112
Hyd 97R 113
Hyd 97R 116
Pant 98 K 1010 X 1011
Pant 98 K 1014 X 1015
M Molecular weight marker
Hyd 97R 112
Hyd 97R 113
Hyd 97R 116
Pant 98 K 1010 X 1011
Pant 98 K 1014 X 1015
M Molecular weight marker

Fig 1 Protein profiles of maize cultivars in SDS-PAGE

Table 2 Seed protein patterns obtained by SDS-PAGE Similarity Matrix in different maize genotypes

Sl.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.
1.	1.00	0.181	0.181	0.147	0.258	0.162	0.135	0.105	0.146	0.1	0.146	0.230	0.264	0.242	0.216	0.15	0.159	0.243	0.297	0.15	0.228
2.		1.00	0.241	0.333	0.241	0.212	.0114	0.083	0.189	0.108	0.189	0.218	0.111	0.210	0.2	0.179	0.230	0.142	0.184	0.162	0.333
3.			1.00	0.2	0.117	0.290	0.083	0.181	0.294	0.108	0.073	0.046	0.081	0.179	0.2	0.194	0.179	0.116	0.125	0.194	0.212
4.				1.00	0.225	0.052	0.026	0.083	0.157	0.051	0.157	0.071	0.052	0.179	0.166	0.228	0.116	0.090	0.097	0.162	0.176
5.					1.00	0.105	0.108	0.078	0.069	0.102	0.045	0.146	0.076	0.2	0.257	0.184	0.086	0.136	0.146	0.153	0.2
6.						1.00	0.162	0.162	0.297	0.153	0.066	0.139	0.189	0.136	0.121	0.175	0.181	0.130	0.139	0.179	0.222
7.							1.00	0.272	0.270	0.222	0.236	0.228	0.194	0.139	0.25	0.121	0.186	0.243	0.170	0.210	0.162
8.								1.00	0.468	0.189	0.146	0.230	0.162	0.195	0.25	0.352	0.214	0.186	0.142	0.095	0.102
9.									1.00	0.166	0.268	0.177	0.142	0.148	0.190	0.342	0.272	0.272	0.204	0.108	0.2
10.										1.00	0.511	0.25	0.153	0.085	0.044	0.2	0.204	0.261	0.25	0.230	0.184
11.											1.00	0.261	0.170	0.125	0.086	0.214	0.302	0.435	0.358	0.243	0.170
12.												1.00	0.441	0.195	0.133	0.181	0.266	0.325	0.384	0.130	0.113
13.													1.00	0.219	0.179	0.068	0.333	0.3	0.324	0.119	0.1
14.														1.00	0.485	0.292	0.260	0.183	0.195	0.177	0.136
15.															1.00	0.289	0.227	0.2	0.214	0.139	0.15
16.																1.00	0.279	0.145	0.238	0.162	0.205
17.																	1.00	0.363	0.425	0.122	0.155
18.																		1.00	0.583	0.25	0.130
19.																			1.00	0.3	0.139
20.																				1.00	0.236
21.																					1.00

1. Pant 98 K (1014 × 1015), 2. Pant 98 K (1010 × 1011), 3. HYD 97 R 116, 4. HYD 97 R 113, 5. HYD 97 R 112, 6. Pant 97 K (1109 × 1108), 7. Pant 97 K (1097 × 1096), 8. Pant 97 K (1091 × 1090), 9. Pant 97 K (1087 × 1086), 10. Pant 97 K (1120 × 1121), 11. Pant 97 K (1115 × 1114), 12. Pant 97 K (1123 × 1124), 13. Pant 97 K (1083 × 1082), 14. Pant 97 K 1046, 15. Pant 97 K 1045, 16. Pant 97 K 1044, 17. Pant 97 K 1043, 18. Pant 97 K 1042, 19. Pant 97 K 1041, 20. Pant 97 K 1040, 21. Pant 97 K 1038

Table 3 Seed protein pattern obtained by PAGE Similarity Matrix in different maize genotypes

Sl.	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.
1.	1.00	0.421	0.260	0.157	0.171	0.070	0.043	0.08	0.136	0.0	0.136	0.0	0.0	0.04	0.148	0.035	0.033	0.190	0.2	0.115	0.064
2.		1.00	0.363	0.210	0.217	0.041	0.041	0.070	0.130	0.040	0.238	0.0	0.0	0.045	0.230	0.071	0.032	0.238	0.25	0.153	0.133
3.			1.00	0.142	0.20	0.24	0.125	0.111	0.070	0.038	0.130	0.034	0.0	0.136	0.096	0.033	0.134	0.166	0.227	0.032	0.2
4.				1.00	0.15	0.043	0.111	0.015	0.105	0.052	0.05	0.0	0.05	0.058	0.038	0.086	0.115	0.166	0.176	0.041	0.115
5.					1.00	0.208	0.136	0.12	0.181	0.040	0.083	0.083	0.183	0.210	0.103	0.071	0.192	0.190	0.25	0.111	0.064
6.						1.00	0.360	0.208	0.385	0.0	0.08	0.08	0.038	0.263	0.064	0.068	0.32	0.08	0.130	0.107	0.064
7.							1.00	0.315	0.437	0.0	0.045	0.094	0.0	0.333	0.035	0.08	0.526	0.15	0.1	0.038	0.148
8.								1.00	0.238	0.040	0.083	0.04	0.0	0.15	0.103	0.111	0.28	0.083	0.136	0.111	0.269
9.									1.00	0.0	0.090	0.090	0.043	0.4	0.0	0.070	0.363	0.2	0.094	0.166	0.032
10.										1.00	0.210	0.210	0.15	0.052	0.02	0.125	0.0	0.0	0.044	0.125	0.24
11.											1.00	0.142	0.2	0.05	0.111	0.12	0.034	0.090	0.15	0.070	0.230
12.												1.00	0.411	0.166	0.111	0.217	0.071	0.090	0.15	0.166	0.066
13.													1.00	0.05	0.071	0.272	0.071	0.090	0.15	0.217	0.103
14.														1.00	0.0	0.041	0.35	0.105	0.111	0.086	0.035
15.															1.00	0.172	0.028	0.034	0.035	0.25	0.114
16.																1.00	0.062	0.12	0.08	0.142	0.2
17.																	1.00	0.153	0.115	0.096	0.151
18.																		1.00	0.277	0.12	0.142
19.																			1.00	0.125	0.111
20.																				1.00	0.005
21.																					1.00

1. Pant 98 K (1014 x 1015), 2. Pant 98 K (1010 x 1011), 3. HYD 97 R 116, 4. HYD 97 R 113, 5. HYD 97 R 112, 6. Pant 97 K (1109 x 1108), 7. Pant 97 K (1097x 1096), 8. Pant 97 K (1091 x 1090), 9. Pant 97 K (1087 x 1086), 10. Pant 97 K (1120 x 1121), 11. Pant 97 K (1115 x 1114), 12. Pant 97 K (1123 x 1124), 13. Pant 97 K (1083 x 1082), 14. Pant 97 K 1046, 15. Pant 97 K 1045, 16. Pant 97 K 1044, 17. Pant 97 K 1043, 18. Pant 97 K 1042, 19. Pant 97 K 1041, 20. Pant 97 K 1040, 21. Pant 97 K 1038

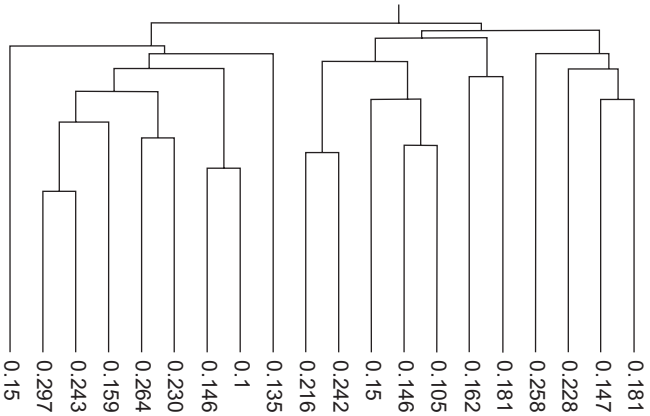


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Fig 2 Dendogram showing genetic relatedness in maize genotypes according to protein profiling in SDS-PAGE using UPGMA method.

58.3% was observed between Pant 97K 1042 and 1041 while 56.2% was highest similarity between Pant 97k 1097 X 1096 and Hyd 97R 113 cultivars in PAGE protein analysis.

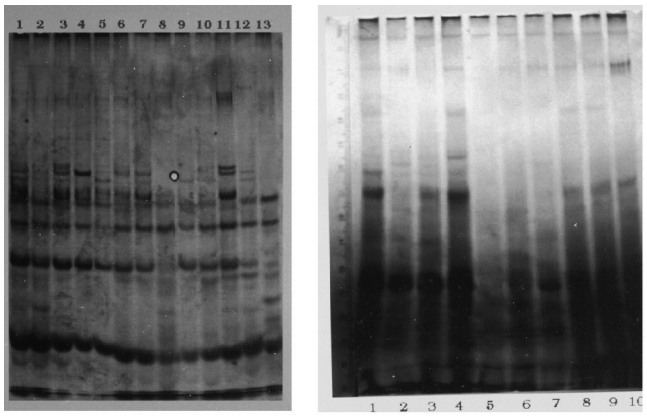


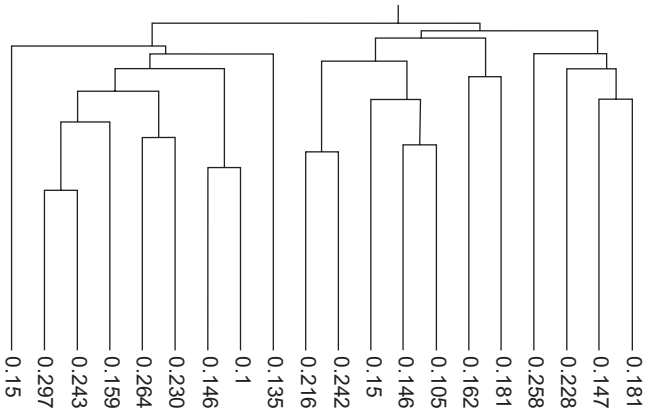
Fig 3 Protein profiles of maize cultivars in Native-PAGE

On the basis of lowest similarity Pant 97K 1097 X 1096 and Hyd 97R 113 in first dendogram (Fig 2) are included in separate major clusters but in native PAGE dendogram (Fig 4) they are included in second major cluster but in two different sub-cluster. Based on highest similarity Pant 97K 1042 and 1041 are showing close resemblance to each other in first dendogram while in native PAGE second dendogram though related to each other are depicted differently in second sub-cluster. The dendogram constructed here revealed the clustering of maize accessions into 2 major clusters which were divided into 4 sub-clusters. Here maize accession Pant 97 K 1040 showed striking difference with others depicted by Pant 97 K 1046 which showed resemblance to some cultivars in Ist cluster and to some accessions in II cluster. This correlation of maize cultivars is also observed in agronomic traits like plant height, cob diameter etc. (Table 1).

The protein based dendogram in native PAGE revealed the clustering of 21 maize accessions into two major clusters which were further divided into two sub cluster each. The clustering of maize cultivars into respective clusters were less related to their pedigree data while some morphological traits were in resemblance to the like ear height, ear type etc.

Genetic diversity of maize germplasm is important for planning breeding programmes, conservation of maize germplasm.

In our study we used protein markers in different condition (i e denaturing and non-denaturing) for



1. Pant 98 K (1014 × 1015), 2. Pant 98 K (1010 × 1011), 3. Hyd 97 R 116, 4. Hyd 97 R 113, 5. Hyd 97 R 112, 6. Pant 97 K (1109 × 1108), 7. Pant 97 K (1097 × 1096), 8. Pant 97 K (1091 × 1090), 9. Pant 97 K (1087 × 1086), 10. Pant 97 K (1120 × 1121), 11. Pant 97 K (1115 × 1114), 12. Pant 97 K (1123 × 1124), 13. Pant 97 K (1083 × 1082), 14. Pant 97 K 1046, 15. Pant 97 K 1045, 16. Pant 97 K 1044, 17. Pant 97 K 1043, 18. Pant 97 K 1042, 19. Pant 97 K 1041, 20. Pant 97 K 1040, 21. Pant 97 K 1038

Fig 4 Dendogram showing genetic relatedness in maize genotypes according to protein profiling in PAGE using UPGMA method.

characterization of maize hybrids and evaluating of there genetic diversity. Maize embryo salt solute proteins are suitable for characterization of maize hybrid as shown previously (Bauer *et al.* 2005). The maize accessions studied here differentiated from each other in both PAGE and SDS-PAGE condition. Here clustering of hybrids based on embryo salt soluble proteins showed good agreement with their pedigree, because hybrids with similar parental components were joined together in smaller groups. These cultivars also showed closed resemblance with agronomic traits like plant height, yield etc. These results are in agreement with Nagy *et al*. (2009) and Abdellatif and Khidr (2010) in maize and EIR Abey *et al*. (2009) in barley cultivars.

The protein profiling done in non-denaturing, i e native PAGE showed less resemblance to pedigree data as compared to protein profiling done in SDS-PAGE. Their resemblance to some morphological traits like ear height, ear type was observed but not consistently suggesting that these traits are not suitable for genetic diversity assessment as these traits are quantitative in nature and they show a large genetic variability. This is in agreement with results of Bauer *et al.* (2005) where embryo salt soluble proteins in non-denaturing condition are not marker system of choice for assessing genetic relatedness of maize hybrids.

Thus it can be concluded that biochemical markers reflect the information of DNA while morphological traits cannot accurately determine the genetic relationships among the different genotypes. SDS-PAGE can be used more effectively to characterize different maize cultivars through seed storage protein electrophoresis among native and SDS-PAGE. It can be concluded that SDS-PAGE either separately or together with agronomic traits is useful for genetic diversity studies in maize.

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