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Research Note

Assessment of genetic diversity in maize (*Zea mays* L.) inbred lines on the basis of soluble seed proteins using SDS-PAGE

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Abstract
In the present investigation 25 genotypes of maize collected from different sources were characterized for the soluble seed proteins using sodium dodecyl sulphate polyacrylamide gelelectrophoresis (SDS-PAGE). The total protein bands were separated on 12% polyacrylamide gel using standard protocols. Highest number of bands observed was 14 in inbred HUZQPM-1.Highest coefficient of similarity matrix was0.69whereas lowest was 0.04. The dendrogram prepared by using UPGMA clustering method separated the whole genotypes into two main groups; I and II. On the basis of present investigation, it is observed that there is low variability present among quality protein maize inbreds.

Keywords
Maize, Genetic diversity, soluble seed protein, SDS-PAGE, Electrophoresis

Maize (*Zea mays* L.) belongs to tribe Maydeae of the Poaceae family. It is the third most important cereal crop in the world as well as in Indiaafter wheat and rice. In India, major quantity of maize grains is utilized as poultry feed (51%) followed by human consumption (23%), animal feed (12%), industrial products (12%) and beverages and starch (1%). In India it is cultivated in the 8.6 million hectare area while its production is 22.2 million tons and productivity is 2566 kg hectare^{-1}(Yadav, 2014) however, productivity of maize in India is nearly half as that of the United States of America (5922 kg hectare^{-1}).Thus, producing new maize hybrids with a high potential of yield production could improve maize productivity in India. Therefore for development of newer highly productive maize hybrids, a critical evaluation of inbred lines is required to assess existing genetic variability (Venugopal et al. 2003)present among them.Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is useful for characterizing, identification and documentation of genetic resources (Konarev*et al.*1979). It is also useful in differentiating and comparing corn endosperm protein fractions (De Freitas *et al.*,2005).Therefore, objective of the present investigation was to assess the genetic diversity present in quality protein maize inbreds by using the electrophoretic profiles of total seed proteins through SDS- PAGE.

Plant material consisted of 25 quality protein maize inbreds which were obtained from obtained from the Varanasi centre of All India Coordinated Maize Improvement Project (Table 1). The Tris soluble proteins were extracted from maize seeds as suggested by Varier*et al.*(1992).The stacking and separating gels were made as suggested by Laemmli (1970).A very small amount of bromophenol blue was added to each sample to avoid mixing of samples in wells and track the movement of samples in gel. The electrophoresis was performed in vertical slab gel type unit. Ten micro litres of each sample were loaded in each well with help of the pipette. Electrophoresis was carried out at a voltage of 100 volts for nearly 3 hours till bromophenol blue dye reached the bottom of the gel. Gels were carefully removed and fixed by immersing them in fixing solution containing 50% methanol, 40% water and 10% acetic acid for 20-30 minutes. The gels were kept overnight in staining solution (ComassieBrilliant Blue R-250) and de-stained used NaCl as suggested by Sreeramulu and Singh (1995). After de-staining, the gels were photographed on a trans-illuminator and analysed for the presence and absence of protein bands. A binary similarity matrix was prepared by scoring for the presence (1) and absence (0) of the corresponding band among the 25 genotypes. The data were used to generate a data matrix in Microsoft Excel 2007. This data matrix was subjected to further analysis using NTSYS-pc version 2.11 (Rohlf, 1997). The SIMQUAL program was used to calculate the Jaccard's similarity coefficients (Jaccard, 1901). The resulting similarity matrix was used to construct UPGMA (UnweightedPair Group Method with Arithmetic Mean) based dendrogram.

The UPGMA based dendrogram was constructed using similarity matrix obtained by using Jaccard's similarity co-efficient.Dendrogram separated 25 quality protein maizeinbreds into two groups (Figure 1). Group I had 24 inbreds while group II had only I inbred HUZQPM-4. The group I was further divided into two clusters A and B. Cluster B

was bigger and had 20 genotypes whereas cluster B had only 4 genotypes. Cluster A was subdivided into two sub-clusters (A1 and A2). Sub-cluster A1 having 8 inbreds namely, HOQC13B-47-B, HKI 193-1, HKI 164-4, CML 147, HOQC13B-32-B, CML 140, HUZQPM-5 and SOISIWQ-2-B-31-B whereas sub-cluster A2 had 12 inbreds viz., HKI 193-2, HUZQPM-7, HOQC13-B-87-B, HUZQPM-3, DMRQPM-58, S99TLWQ-1-B-2-B, HUZQPM-6, SOISIWQ-2B-43-B, DMRQPM-60, HOQC13-B-29-B, SSO2O2C(Y)-B-15-Band HOQC13-B-82-B. Seed protein electrophoresis was also used by Ladizinsky and Hymowithz (1979) for taxonomic studies. On the basis of similarity coefficients it can be concluded that Inbred SOISIWQ-2-B-43-B and DMRQPM-60 (69%) had maximum similarity where as inbred DMRQPM-60 and HUZQPM-4 (4%) had minimum similarity (Table 2). Similar findings were also reported by Nagy *et al.* (2009).

Thus, on the basis of the present study, it can be stated that biochemical markers especially seed protein markers can be used more effectively to characterize different maize cultivars through SDS-PAGE. It is obvious that the elucidation of genetic diversity is most important for evaluation, maintenance and designing a successful hybrid development programme. Although 25 quality protein maize genotypes used in present investigation had does not have sufficient genetic diversity, therefore, there is a constant need for introgressionof QPM genes in diverse backgrounds.

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Table 1. List of 25 genotypes used in present study

Genotype No.	Name of Genotype	Origin
1.	HOQC13B-47-B	Directorate of Maize Research, New Delhi
2.	HKI 193-1	C.C.S. Hisar Agriculture University, Haryana
3.	HOQC13B-32-B	Directorate of Maize Research, New Delhi
4.	CML-140	CIMMYT-Mexico
5.	HUZQPM-5	Banaras Hindu University, Varanasi
6.	SOISIWQ-2-B-31-B	Directorate of Maize Research, New Delhi
7.	HKI 193-2	C.C.S. Hisar Agriculture University, Haryana
8.	CML-147	CIMMYT-Mexico
9.	HUZQPM-1	Banaras Hindu University, Varanasi
10.	HUZQPM-8	Banaras Hindu University, Varanasi
11.	SSO2O2C(Y)-B-28-B	Directorate of Maize Research, New Delhi
12.	HUZQPM-7	Banaras Hindu University, Varanasi
13.	HOQC13-B-87-B	Directorate of Maize Research, New Delhi
14.	S99TLWQ-1-B-2-B	Directorate of Maize Research, New Delhi
15.	SSO2O2C(Y)-B-15-B	Directorate of Maize Research, New Delhi
16.	SOISIWQ-2-B-43-B	Directorate of Maize Research, New Delhi
17.	DMRQPM-60	Directorate of Maize Research, New Delhi
18.	HUZQPM-6	Banaras Hindu University, Varanasi
19.	HOQC13-B-29-B	Directorate of Maize Research, New Delhi
20.	DMRQPM-58	Directorate of Maize Research, New Delhi
21.	HUZQPM-3	Banaras Hindu University, Varanasi
22.	HOQC13-B-16-B	Directorate of Maize Research, New Delhi
23.	HUZQPM-4	Banaras Hindu University, Varanasi
24.	HOQC13-B-82-B	Directorate of Maize Research, New Delhi
25.	HKI 164-4	C.C.S. Hisar Agriculture University, Haryana

Table 2. Similarity matrix among 25 genotypes using SDS-PAGE

G.No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1.00																								
2	0.50	1.00																							
3	0.25	0.20	1.00																						
4	0.18	0.14	0.43	1.00																					
5	0.11	0.19	0.17	0.40	1.00																				
6	0.11	0.33	0.18	0.18	0.31	1.00																			
7	0.19	0.15	0.06	0.12	0.18	0.12	1.00																		
8	0.19	0.28	0.19	0.12	0.18	0.27	0.06	1.00																	
9	0.04	0.04	0.20	0.09	0.04	0.14	0.10	0.15	1.00																
10	0.09	0.12	0.20	0.14	0.14	0.20	0.10	0.15	0.56	1.00															
11	0.06	0.05	0.36	0.19	0.05	0.12	0.06	0.06	0.35	0.35	1.00														
12	0.17	0.06	0.08	0.17	0.25	0.08	0.30	0.18	0.06	0.06	0.08	1.00													
13	0.14	0.11	0.14	0.33	0.13	0.07	0.25	0.07	0.11	0.05	0.15	0.25	1.00												
14	0.25	0.14	0.11	0.11	0.11	0.05	0.27	0.12	0.14	0.09	0.06	0.27	0.33	1.00											
15	0.27	0.15	0.06	0.12	0.11	0.06	0.29	0.20	0.10	0.10	0.06	0.30	0.25	0.46	1.00										
16	0.11	0.14	0.11	0.18	0.11	0.11	0.19	0.27	0.20	0.14	0.19	0.17	0.45	0.43	0.36	1.00									
17	0.16	0.18	0.22	0.22	0.21	0.16	0.17	0.24	0.18	0.13	0.11	0.14	0.38	0.47	0.31	0.69	1.00								
18	0.18	0.09	0.11	0.11	0.17	0.05	0.36	0.12	0.14	0.09	0.06	0.27	0.33	0.67	0.36	0.43	0.57	1.00							
19	0.13	0.05	0.21	0.21	0.13	0.06	0.23	0.07	0.17	0.05	0.14	0.22	0.30	0.42	0.23	0.31	0.36	0.31	1.00						
20	0.05	0.04	0.10	0.21	0.14	0.05	0.16	0.16	0.17	0.17	0.16	0.13	0.36	0.28	0.22	0.44	0.47	0.44	0.25	1.00					
21	0.11	0.14	0.18	0.33	0.24	0.18	0.19	0.06	0.14	0.20	0.12	0.17	0.45	0.18	0.12	0.25	0.29	0.18	0.21	0.44	1.00				
22	0.11	0.14	0.11	0.18	0.24	0.11	0.36	0.19	0.09	0.20	0.12	0.17	0.33	0.33	0.36	0.43	0.47	0.43	0.21	0.35	0.25	1.00			
23	0.10	0.17	0.15	0.10	0.09	0.05	0.16	0.16	0.13	0.13	0.16	0.13	0.12	0.10	0.10	0.05	0.04	0.10	0.05	0.24	0.21	0.05	1.00		
24	0.18	0.20	0.11	0.18	0.24	0.11	0.19	0.06	0.20	0.20	0.12	0.08	0.23	0.11	0.12	0.18	0.22	0.18	0.13	0.15	0.18	0.25	0.05	1.00	
25	0.35	0.35	0.15	0.15	0.26	0.15	0.10	0.22	0.08	0.13	0.05	0.13	0.12	0.21	0.16	0.10	0.19	0.15	0.11	0.08	0.15	0.15	0.18	0.28	1.00

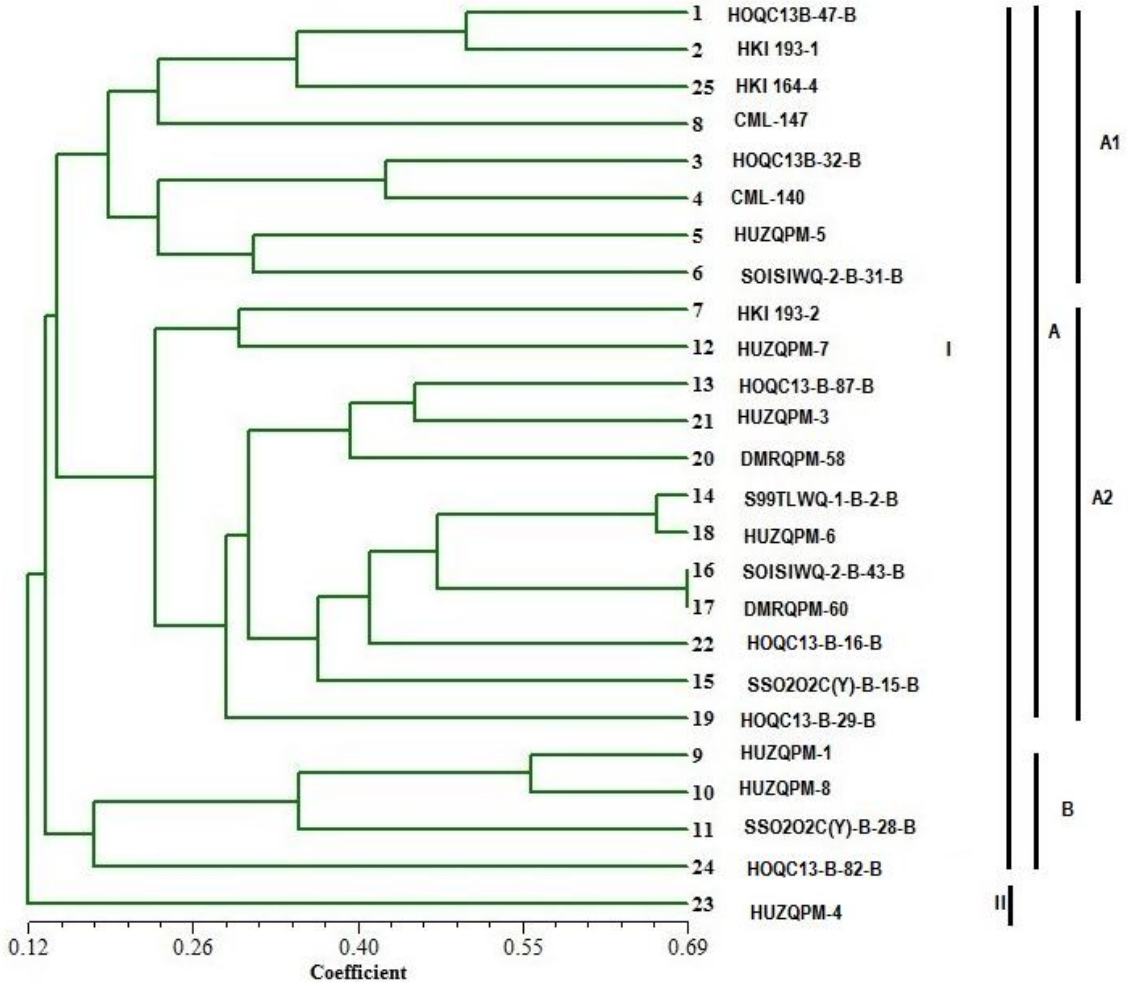

Figure 1. Dendrogram showing the relationship among 23 maize inbred lines.