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Research Note

Characterization of pearl millet hybrids and their parental lines by seed storage protein markers

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Abstract
The variability of seed storage-proteins of 21 pearl millet genotypes were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophorogram for 21 Pearl millet genotypes (seven hybrids, six female parent, five male parent and three Open Pollinated Variety(OPV)) were scored and Jaccard's similarity index (JSI) was calculated. Genetic diversity of Pearl millet genotypes was evaluated by constructing the dendrogram with Unweighted Pair Group Method Arithmetic Average (UPGMA) algorithm using NTSYSPC version 2.01 software. Six mobility bands that were polymorphic (Rm=0.33, 0.39, 0.43, 0.51, 0.69 and 0.75) were used for genotyping. Though it proved an efficient method to distinguish a few genotypes (H 90/4-5, H77/833-2, G 73-107, H 77/29-2, ICMA 95222A, HHB 146, MS 843A and ICMA 89111A) based on banding pattern. Intensity of bands were also some extant helped to differentiate genotypes especially Band with Rm value of 0.43 was light for H 77/29-2, band with Rm value of 0.51 was very light for G73-107 and band with Rm value of 0.75 was medium intensity for H 90/4-5. It is concluded that seed storage protein profiles could be useful markers in the studies of genetic diversity and classification of Pearl millet genotypes.

Key words
Pearl millet varieties, SDS-PAGE, genetic diversity and cluster analysis.

Proteins are attractive for direct genetic study because they are primary products of structural genes. Seed Proteins are biological mixture of several proteins and separation of each individual polypeptide of a protein mixture has its own optimum pH point. Thus proteins from the seeds of different plant separate best at specific pH values during electrophoresis. The most common system of electrophoresis appears to be the sodium dodecyl sulfate polyacrylamide system developed by Laemmli(1970). Polyacrylamide gel electrophoresis of total seed protein has been widely investigated as a method for varieties identification in various crops (Shuaib *et al.*, 2007). Seed storage protein analysis represents a valid alternative and/or improved approach to varietal identification, which currently is based on morphological traits recorded in the field (AOSA, 1991). Seed storage protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Gepts *et al.*,1986). Morphometric and seed protein analysis were used to reveal differences among species. Total soluble protein markers are also used extensively in characterization of rye grass (Gustine *et al.*1996) and vegetables (Mollema and Cole, 1996). In Pearl millet several genotypes have been identified with help of electrophorogram of seed protein (Varier *et al.*1990).Present study was envisaged to characterize 21 Pearl millet genotypes by using protein profiling.

Seven released pearl millet hybrids from CCS HAU along with their parental lines were characterized using their protein profiling. Seven hybrids *viz.*, HHB 50[H1] (MS 81A × H 90/4-5), HHB 60[H2] (MS 81A × H 77/833-2), HHB 67[H3] (MS 843A × H 77/833-2), HHB 68[H4] (MS 842A × H 77/833-2), HHB 94[H5] (ICMA 89111A × G 73-107), HHB 117[H6] (HMS 7A × H 77/29-2) and HHB 146[H7](ICMA 95222A × HTP 94/54), Six male sterile lines *viz.*, MS 81A[F1/F2], MS 843A[F3], MS 842A[F4], ICMA 89111A[F5], HMS 7A[F6] and ICMA 95222A[F7], Five restorer lines *viz.*, H 90/4-5[M1], H 77/833-2[M2/M3/M4], G 73-107[M5], H 77/29-2[M6] and HTP 94/54[M7] and three Open pollinated varieties *viz.*, HC 4 [OPV1] , HC 10[OPV2] and HC 20[OPV3]. These 21 genotypes comprised the experimental materials for the present study (Table 1). The experiment was conducted at Department of Plant Breeding, College of Agriculture, CCS HAU, Hisar.

For studying polypeptite pattern, denaturing discontinuous polyacrylamide gel electrophoresis method of Lamelli (1970) was followed. Electrophoresis was carried out using a Hoefer mini gel unit applying a constant current of 40 mA until the tracking dye, bromophenol blue reached the bottom. The gels were taken out and stained using Coomasie blue stain solution overnight and destained using methanol (40 %) and acetic acid (7 %) solutions. The electrophorograms were prepared on the basis of protein mobility and the

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density expressed. Based on polyacrylamide gel, bands were scored as present (1) and absent (0) in data sheet to form a (1, 0) matrix. Then data were analyzed and similarity matrix was constructed from binary data with Jaccard's coefficient (Jaccard,1908) and dendrogram were generated with Unweighted Pair Group Method Arithmetic Average (UPGMA) algorithm using NTSYSPC – version 2.01 software (Rholf, 2000).

Twelve bands were found with Rm value ranges from 0.33-0.99(Table 2). Bands with Rm values of 0.58, 0.81, 0.88, 0.90, 0.92 and 0.99 were found to be monomorphic for all genotypes. Another six bands with Rm values of 0.33, 0.39, 0.43, 0.51, 0.69 and 0.75 were polymorphic in nature. Medium intensity band with Rm value of 0.33 (Table 3) was present in HHB 50(H1), HHB 60(H2), HHB 68(H4), HHB 94(H5), HHB 117(H6), MS 81A (F1/F2), MS 842A (F4), H77/833-2(M2/M3/M4). Band with Rm value of 0.39 was absent for H90/4-5(M1), G 73-107 (M5),HTP 94/54(M7), but medium intensity of band was found for H77/833-2(M2/M3/M4) and rest of the other genotypes shown light intensity of band. Band with Rm value 0.43 was absent for H 90/4-5(M1),H77/833-2(M2/M3/M4),G73-07(M5), HTP 94/54(M7), MS 843A(F3), HHB67 (H3) and HHB 68(H4), Hybrids HHB 94(H5), HHB 117(H6), HHB 146(H7) with their female parents ICMA 89111A(F5), HMS7A(F6), ICMA 95222A(F7) were shown medium intensity of bands, Hybrids HHB 50(H1), HHB60 (H2) and their female parent MS 81A (F1/F2) were showed dark intensity of bands. Light intensity of band with Rm value of 0.43 was found for H77/29-2(M6) only. Band with Rm value of 0.51 was absent for H77/833-2(M2/M3/M4) and very light intensity for G73-107 (M5) genotype. Rm value of 0.69 band was absent for G73-107(M5), HTP94/54(M7), ICMA89111A (F5), HHB50 (H1). Medium intensity band with Rm value 0.75 was found for H90/4-5(M1) and absent for G73-107(M5),H77/29-2(M6), HTP 94/54(M7), MS843A(F3), ICMA89111A(F5), ICMA95222A (F7), HHB 67(H3), HHB 147(H7) and other genotypes showed dark intensity of bands. All genotypes could be grouped into as much as four clusters based on less than 50% Jaccard's similarity coefficient (Fig.1). H90/4-5(M1), G73-107(M5), HTP94/54(M7) were formed cluster one. Second cluster consisted of three sub clusters viz., HMS7A (F6), H77/29-2(M6), ICMA 95222A (F7), HHB147 (H7) of one sub clusters, HHB67 (H3) and MS843A (F3) of another sub cluster and ICMA89111A (F5) alone formed third sub cluster. Third cluster consisted of HHB 68 (H4) and H77/833-2 (M2/M3/M4). Hybrids HHB50 (H1), HHB 60(H2) with their female parent MS 81A (F1/F2), hybrids HHB 94 (H5), HHB117 (H6) and MS 842A (F4) formed fourth cluster. It was found that in each cluster, the members were 100% similar to each other. Besides the similarity coefficients (Table 4) ranged from 0.5833 to 0.9167.

Hybrids HHB50 (H1), HHB 67 (H3) and HHB147 (H7) closely resembled (SI=1.0000) (Table 5) with their female parents MS81A (F1), MS 843A (F3) and ICMA 95222A (F7). Hybrids HHB 60(H2), HHB 68(H4) and HHB117 (H6) closely resembled (SI=0.9167) with their female parents viz., MS 81A (F2), MS842A (F4) and HMS 7A (F6). Hybrid HHB 94(H5) moderately resembled (SI=0.7500) with its female parent ICMA 89111A (F5).

Genetic purity testing is carried out to ensure seed quality which is imperative for efficient crop production. Seed testing programmes need quick, economic and reliable methods to keep pace with release of new varieties every year. Generally morphological characters suggested by breeders are used to check the genetic purity of seed lots. These features mainly include floral characters which take a long time for their expression to be observed. Moreover the results of grow out test used for genetic purity are applicable next year of conducting it, thus slowing down the process of varietal seed production programmes. Therefore, novel methods, which are quick and easily reproducible, are required. Electrophoresis is one of such techniques, which has been extensively used for identification of varieties and their genetic purity by many workers in different crops such as wheat, barley, oat, rice etc. Varier *et al.*, 1990 used it in pearl millet for varietal identification. Kumar *et al*.(1995) and Chhabra *et al*.(1996) also used in this techniques in Pearl millet.

In the present study also, the lower molecular weight protein bands (Rm = 0.58, 0.81, 0.88, 0.90, 0.92 and 0.99) were of no use being monomorphic in nature. Therefore only a few mobility bands that were polymorphic (Rm=0.33, 0.39, 0.43, 0.51, 0.69 and 0.75) were used for genotyping. Though it proved an efficient method to distinguish a few genotypes (H 90/4-5, H77/833-2, G 73-107, H 77/29-2, ICMA 95222A, HHB 146, MS 843A and ICMA 89111A) based on banding pattern. Intensity of bands were also some extant helped to differentiate genotypes especially Band with Rm value of 0.43 was light for H 77/29-2, band with Rm value of 0.51 was very light for G73-107 and band with Rm value of 0.75 was medium intensity for H 90/4-5. In conclusion, characterization of these hybrids and their parental lines was done based on their seed storage protein profiling only, for accurate molecular characterization can be possible by using DNA based molecular markers.

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Table 1. List of Pearl millet genotypes and their pedigree

S.No	Genotype	Code	Status	Pedigree	Year of Release	Origin
1	HHB 50	H1	Hybrid	MS 81A × H 90/4-5	1987	CCS HAU, Hisar
2	HHB 60	H2	Hybrid	MS 81A × H 77/833-2	1988	CCS HAU, Hisar
3	HHB 67	H3	Hybrid	MS 843A × H 77/833-2	1990	CCS HAU, Hisar
4	HHB 68	H4	Hybrid	MS 842A × H 77/833-2	1993	CCS HAU, Hisar
5	HHB 94	H5	Hybrid	ICMA 89111A × G 73-107	1999	CCS HAU, Hisar
6	HHB 117	H6	Hybrid	HMS 7A × H 77/29-2	2002	CCS HAU, Hisar
7	HHB 146	H7	Hybrid	ICMA 95222A × HTP 94/54	2002	CCS HAU, Hisar
8	MS 81A	F1/F2	CMS	Derived from Tift $23D_2$ after irradiation	1981	ICRISAT, Hyderabad
9	MS 843A	F3	CMS	Selected from AKM 2068 for Downy mildew resistance	1984	ICRISAT, Hyderabad
10	MS 842A	F4	CMS	Re Selected from AKM 2068 for Downy mildew resistance	1984	ICRISAT, Hyderabad
11	ICMA 89111A	F5	CMS	881A cytoplasm source(B_1) backcrossed to ICMB 89111	1989	ICRISAT, Hyderabad
12	HMS 7A	F6	CMS	Developed by backcrossing from the cross 81 A × 35(81B × 69B)	1991	ICRISAT, Hyderabad
13	ICMA 95222A	F7	CMS	81A cytoplasm(A_1) source back crossed to ICMB 95222	1995	ICRISAT, Hyderabad
14	H 90/4-5	M1	Restorer	Developed by selecting selfed progenies form synthetic HSI	1976	CCS HAU, Hisar
15	H 77/833-2	M2/M3/M4	Restorer	Developed by selfing a Haryana land race population	1976	CCS HAU, Hisar
16	G 73-107	M5	Restorer	Developed by selecting selfed progenies of GAM 73	1976	CCS HAU, Hisar
17	H 77/29-2	M6	Restorer	Developed by selecting selfed plants from Rajasthan landrace	1976	CCS HAU, Hisar
18	HTP 94/54	M7	Restorer	Developed by selecting selfed progenies of high tillering of Tago population	1992	CCS HAU, Hisar
19	HC 4	OPV1	Open pollinated variety	Developed by intermating seven inbred lines	1985	CCS HAU, Hisar
20	HC 10	OPV2	Open pollinated variety	Bred by random mating 15 S1 progenies of NELC population	1999	CCS HAU, Hisar
21	HC 20	OPV3	Open pollinated variety	Bred by random mating S1 progenies from gene pool selected for good yield and drought stress	2000	CCS HAU, Hisar

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Table 2. Banding pattern of tris-soluble total seed storage protein in 21 genotypes of Pearl millet

Bands	Rf/Rm	M1	M2	M3	M4	M5	M6	M7	F1	F2	F3	F4	F5	F6	F7	H1	H2	H3	H4	H5	H6	H7
1	0.33	-	+	+	+	-	-	-	+	+	-	+	-	-	-	+	+	-	+	+	+	-
2	0.39	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	0.43	-	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	-	-	+	+	+
4	0.51	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	0.58	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	0.69	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	-	+	+	+	+	+
7	0.75	+	+	+	+	-	-	-	+	+	-	+	-	+	-	+	+	-	+	+	+	-
8	0.81	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	0.88	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	0.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	0.92	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	0.99	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ Presence of band, - Absence of band, H-hybrid, M- male parent, F-female parent

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Table 3. Schematic Zymogram of tris-soluble total seed storage protein in 21 genotypes of Pearl millet

Bands	Rf/Rm	M1	M2	M3	M4	M5	M6	M7	F1	F2	F3	F4	F5	F6	F7	H1	H2	H3	H4	H5	H6	H7
1	0.33	-	M	M	M	-	-	-	M	M	-	M	-	-	-	M	M	-	M	M	M	-
2	0.39	-	M	M	M	-	L	-	L	L	L	L	L	L	L	L	L	L	L	L	L	L
3	0.43	-	-	-	-	-	L	-	D	D	-	D	M	M	M	D	D	-	-	M	M	M
4	0.51	L	-	-	-	VL	D	L	D	D	D	D	M	M	M	D	D	D	D	M	M	M
5	0.58	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
6	0.69	D	D	D	D	-	D	-	D	D	D	D	-	D	D	D	-	D	D	D	D	D
7	0.75	M	D	D	D	-	-	-	D	D	-	D	-	D	-	D	D	-	D	D	D	-
8	0.81	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
9	0.88	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
10	0.9	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
11	0.92	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
12	0.99	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

D-Dark, M-Medium, L-Light, VL-Very light, - Absent - Absence of band, H_x-hybrid, M_x- male parent, F_x-female parent

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Table 4. Similarity Indices among 21 genotypes of Pearl millet based on tris soluble total seed storage protein.

	M1	M2	M3	M4	M5	M6	M7	FI	F2	F3	F4	F5	F6	F7	HI	H2	H3	H4	H5	H6	H7
M1	1.00																				
M2	0.75	1.00																			
M3	0.75	1.00	1.00																		
M4	0.75	1.00	1.00	1.00																	
M5	0.83	0.58	0.58	0.58	1.00																
M6	0.75	0.67	0.67	0.67	0.75	1.00															
M7	0.83	0.58	0.58	0.58	1.00	0.75	1.00														
FI	0.75	0.83	0.83	0.83	0.58	0.83	0.58	1.00													
F2	0.75	0.83	0.83	0.83	0.58	0.83	0.58	1.00	1.00												
F3	0.83	0.75	0.75	0.75	0.83	0.92	0.83	0.75	0.75	1.00											
F4	0.75	0.83	0.83	0.83	0.58	0.83	0.58	1.00	1.00	0.75	1.00										
F5	0.67	0.58	0.58	0.58	0.83	0.92	0.83	0.75	0.75	0.83	0.75	1.00									
F6	0.83	0.75	0.75	0.75	0.67	0.92	0.67	0.92	0.92	0.83	0.92	0.83	1.00								
F7	0.75	0.67	0.67	0.67	0.75	1.00	0.75	0.83	0.83	0.92	0.83	0.92	0.92	1.00							
HI	0.75	0.83	0.83	0.83	0.58	0.83	0.58	1.00	1.00	0.75	1.00	0.75	0.92	0.83	1.00						
H2	0.67	0.75	0.75	0.75	0.67	0.75	0.67	0.92	0.92	0.67	0.92	0.83	0.83	0.75	0.92	1.00					
H3	0.83	0.75	0.75	0.75	0.83	0.92	0.83	0.75	0.75	1.00	0.75	0.83	0.83	0.92	0.75	0.67	1.00				
H4	0.83	0.92	0.92	0.92	0.67	0.75	0.67	0.92	0.92	0.83	0.92	0.67	0.83	0.75	0.92	0.83	1.00	1.00			
H5	0.75	0.83	0.83	0.83	0.58	0.58	1.00	1.00	0.75	1.00	0.75	0.92	0.83	1.00	0.92	0.75	0.92	0.92	1.00		
H6	0.75	0.83	0.83	0.83	0.58	0.58	1.00	1.00	0.75	1.00	0.75	0.92	0.83	1.00	0.92	0.75	0.92	0.92	1.00	1.00	
H7	0.75	0.67	0.67	0.67	0.75	1.00	0.75	0.83	0.83	0.91	0.83	0.92	0.92	1.00	0.83	0.75	0.92	0.75	0.83	0.83	1.00

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Table 5. Similarity indices between hybrid and their parents

Hybrid	Female Parent	Male Parent
HHB 50 (H1)	1.00	0.75
HHB 60 (H2)	0.91	0.75
HHB 67 (H3)	1.00	0.75
HHB 68 (H4)	0.91	0.91
HHB 94 (H5)	0.75	0.58
HHB 117 (H6)	0.91	0.83
HHB 146 (H7)	1.00	0.75

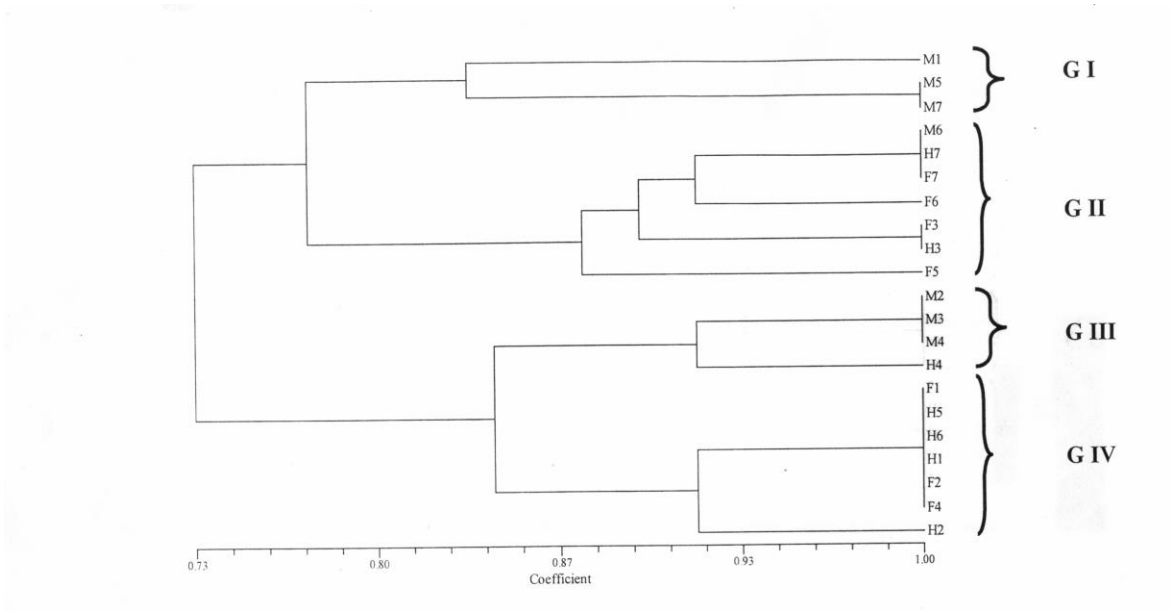

Fig.1. Dendrogram of 21 genotypes of Pearl millet based on tris soluble total seed storage protein