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Peroxidase isozyme characterization of elite genotypes of Pearl millet (*Pennisetum glaucum* (L.) R. Br)

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Isozyme markers are the oldest among molecular markers. Isozyme markers have been successfully used in several crop improvement programmes. Peroxidase (POX) isozyme has proven to be reliable genetic marker in breeding and genetic studies of Pearl millet. The present study was conducted to characterize 21 genotypes (7 hybrids, 6 female parents, 5 male parents and 3 open pollinated varieties (OPV) of Pearl millet by using POX isozyme. Five bands were found with Rm/Rf value ranges from 0.5 to 0.64. All bands were found to be polymorphic in nature except band with Rm value of 0.55 which was present in all genotypes. Intensity of bands varied with each genotype. Only 3 genotypes (H 77/29-2, HMS 7A and HHB 94) out of 21 genotypes were differentiated from other genotypes. Similarity indices based on POX banding pattern revealed that hybrid HHB 50, HHB 60, HHB 67 and HHB 146 completely resembled (SI 1.000) with their female parents MS 81A, MS 843A and ICMA 95222A. This showed the maximum contribution of female parent in comparison with male parent towards the development of hybrid.

Key words: Peroxidase, characterization, banding pattern.

INTRODUCTION

Pearl millet (*Pennisetum glauccum* (L.) R. Br.) provides stable food for millions of people of African countries and Indian sub-continent. It is the sixth important cereal, primarily gown for grain and fodder production. Pearl millet growing in environments in these areas are characterized by low and erratic rainfall, high temperature and poor soil fertility. In these environments, Pearl millet is the only successful cereal and a major source of energy for the poor farming community. With ability to adopt diverse agro-ecological conditions, it plays a unique position in world agriculture. Pearl millet is a summer annual grass originating from Africa, from where it was introduced into other regions of the world with diverse agro-climatic conditions, that is, from the hot area of Africa to the hot area of temperate zones. Therefore, a large number of diversity is found within and among pearl millet cultivars. Due to its highly out-crossing breeding behaviour, Pearl millet was originated from several independent domestication events and wide range of stressful environmental conditions, in which it had been traditionally cultivated. Pearl millet exhibits a tremendous amount of diversity at both phenotypic and genotypic levels (Poncet et al., 1998; Liu et al., 1994).

Estimation of genetic diversity and identification of superior genotypes are some of the prime objectives of any crop improvement programmes. Highly diverse genotypes or accessions can be utilized as parents in hybridization programmes to produce superior varieties/hybrids. Therefore, there is a need to evaluate available genotypes for their genetic diversity. In the early

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days, crop breeders used morphological markers for the assessment of genetic diversity and choosing parents for developing new cultivars. Morphological markers data are affected by the interaction of the genotype with the environment in which it is expressed. Moreover, due to the high out-crossing breeding nature and structure of genetic diversity in pearl millet species, the morphological data/markers are inadequate in providing reliable information for the calculation of genetic distance and pedigree studies.

Isozyme markers are the oldest among the molecular markers. Isozyme markers have been successfully used in several crop improvement programmes (Glaszmann et al., 1989; Baes and Custsem, 1993). Isozymes have proven to be reliable genetic markers in breeding and genetic studies of plant species (Heinz, 1987), due to consistency in their expression, irrespective of environmental factors. Isozymes provide useful evidences in the study of variation between cultivars in terms of intensity of common bands and presence or absences of other bands (William and Mujeeb, 1992). Study of the isozyme pattern is considered as an important tool for understanding the genetic relationship between individuals and also for the identification of hybrids. Genetically, the production of isozyme of multiple forms or molecular weight is accounted to the allelic variation of the organism. Therefore, isozymes of a particular molecular weight can be considered as a direct manifestation of the blue print of the specific gene loci (Abiden and Vijayakumar, 2002). The utility of isozymes as genetic marker (Cheniany, 2007) is generally attributed to their polymorphism, codominence, simple inheritance, simple assay and obliquity in plant tissues or organs (Simpson and Withers, 1986). Moreover, isozymes study may be useful to diversity analysis in plants (Philomina and Surendran, 2003). Peroxidase (POX) isozyme has been widely used for characterization of plant germplasm (Li and Li, 1996; Ju-Zheng et al., 1997; Gupta et al., 2008). An attempt was made to study the isozyme diversity in some of elite Pearl millet varieties, hybrids and their parental lines.

MATERIALS AND METHODS

Seven (7) 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 (6) 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 (5) 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 (3) open pollinated varieties (OPV) viz., HC 4[OPV1], HC 10[OPV2] and HC 20[OPV3]. These 21 genotypes comprised the experimental materials for the present study (Table 1).

To record the electrophoregram of POX, the method followed was that of Mitra et al. (1970). The POX was displayed as brown bands. 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 (Rohlf, 2000).

RESULTS AND DISCUSSION

Five bands were found with Rm/Rf value ranges from 0.5 to 0.64 (Table 2). All bands were found to be polymorphic in nature except band with Rm value of 0.55 which was present in all genotypes. Intensity of bands varied with each genotype (Table 3). Band of Rm value 0.5 showed dark band for MS 842A(F4), HHB68(H4) and HHB94(H5), medium intensity for HHB 60(H2), very light for MS81A(F1/F2), HMS 7A(F6) and HHB 50(H1). Band at Rm value 0.52 showed medium intensity for H 90/4-5(M1), MS 843(F3), ICMA 95222A(F7), HHB 67(H3), HHB 146(H7), dark for H 77/833-2 (M2/M3/M4), G 73/107(M5), H 77/29-2 (M6), MS 81A(F1/F2), MS 842 A(F4), ICMA 89111A(F5), HHB 50(H1), HHB 68(H4), HHB 117 (H6) and light for HTP 94/54(M7) and HHB 60(H2). Band at Rm value of 0.55 showed dark intensity for G 73-107(M5), H 77/29-2(M6), MS81A(F1/F2), ICMA 89111A(F5), HMS 7A(F6), ICMA 95222A(F7), HHB 50(H1), HHB 94(H5), HHB 117(H6), medium intensity for H 90/4-5(M1), MS 842A(F4), HHB 68(H4), and light intensity for H77/833-2 (M2/M3/M4), ICMA 95222A(F7), MS843A(F3), HHB 60(H2), HHB 67(H3). Band at Rm value of 0.61 showed dark for ICMA 89111A(F5), ICMA 95222A(F7), HHB94(H5), HHB146(H7), medium intensity for H77/833-2(M2/M3/M4), G73-107(M5), HHB68 (H4), HHB117(H6), light for HTP94/54(M7), MS81A(F1/F2), MS843A(F3), HHB50(H1), HHB60(H2), HHB67(H3) and very light intensity for H90/4-5(M1). Band at Rm value of 0.64 showed dark intensity for HMS 7A(F6), HHB 117(H6), medium intensity for ICMA 89111A(F5), HHB 94(H5), light intensity for H77/833-2(M2/M3/M4), G73-107(M5), H77/29-2(M6), HTP 94/54(M7), MS 81A(F1/F2), MS 843A(F3), ICMA 95222A(F7), HHB 50(H1), HHB 60(H2), HHB 67(H3) and very light intensity for H 90/4-5(M1)and HHB 146(H7). Out of 21 genotypes, only 3 genotypes [H 77/29-2(M6), HMS 7A(F6) and HHB 94(H5)] were differentiated from other genotypes. H77/29-2(M6) showed the presence of band with Rm value of 0.52 but absence of other polymorphic bands (Rm = 0.50, 0.61). HMS 7A(F6) had shown only one of these three bands (Rm = 0.50), whereas a band of Rm = 0.52 value was absent in HHB 94 and having other two bands (Rm = 0.50 and 0.61). Based on zymogram, HHB 60(H2) showed light band at RM value of 0.5. H90/4-5(M1) showed very light band at Rm value of 0.61. Band of 0.52 RM value showed light band for HTP 94/54(M7) and HHB 60(H2). Very light band was observed at RM value of 0.64 for H 90/4-5(M1) and HHB 146(H7).

Table 1. List of Pearl millet genotypes and their pedigree.

S/N	Genotype	Status	Pedigree	Year of release	Origin
1	HHB 50	Hybrid	MS 81A × H 90/4-5	1987	CCS HAU, Hisar
2	HHB 60	Hybrid	MS 81A × H 77/833-2	1988	CCS HAU, Hisar
3	HHB 67	Hybrid	MS 843A × H 77/833-2	1990	CCS HAU, Hisar
4	HHB 68	Hybrid	MS 842A × H 77/833-2	1993	CCS HAU, Hisar
5	HHB 94	Hybrid	ICMA 89111A × G 73-107	1999	CCS HAU, Hisar
6	HHB 117	Hybrid	HMS 7A × H 77/29-2	2002	CCS HAU, Hisar
7	HHB 146	Hybrid	ICMA 95222A × HTP 94/54	2002	CCS HAU, Hisar
8	MS 81A	CMS	Derived from Tift $23D_2$ after irradiation	1981	ICRISAT, Hyderabad
9	MS 843A	CMS	Selected from AKM 2068 for Downy mildew resistance	1984	ICRISAT, Hyderabad
10	MS 842A	CMS	Re Selected from AKM 2068 for Downy mildew resistance	1984	ICRISAT, Hyderabad
11	ICMA 89111A	CMS	881A cytoplasm source(B_1) backcrossed to ICMB 89111	1989	ICRISAT, Hyderabad
12	HMS 7A	CMS	Developed by backcrossing from the cross 81 A × 35(81B × 69B)	1991	ICRISAT, Hyderabad
13	ICMA 95222A	CMS	81A cytoplasm(A_1) source back crossed to ICMB 95222	1995	ICRISAT, Hyderabad
14	H 90/4-5	Restorer	Developed by selecting selfed progenies form synthetic HSI	1976	CCS HAU, Hisar
15	H 77/833-2	Restorer	Developed by selfing a Haryana land race population	1976	CCS HAU, Hisar
16	G 73-107	Restorer	Developed by selecting selfed progenies of GAM 73	1976	CCS HAU, Hisar
17	H 77/29-2	Restorer	Developed by selecting selfed plants from Rajasthan landrace	1976	CCS HAU, Hisar
18	HTP 94/54	Restorer	Developed by selecting selfed progenies of high tillering of Tago population	1992	CCS HAU, Hisar
19	HC 4	OPV	Developed by intermating seven inbred lines	1985	CCS HAU, Hisar
20	HC 10	OPV	Bred by random mating 15 S1 progenies of NELC population	1999	CCS HAU, Hisar
21	HC 20	OPV	Bred by random mating S1 progenies from gene pool selected for good yield and drought stress	2000	CCS HAU, Hisar

Table 2. Banding pattern of POX isozyme in 21 genotypes of pearl millet.

Band	Rf/Rm	H 90/4-5	H 77/833-2	H 77/833-2	H 77/833-2	G 73-107	H 77/29-2	HTP 4/54	MS 81A	MS 81A	MS 843A	MS 842A	ICMA 89111A	HMS 7A	ICMA 95222A	HHB 50	HHB 60	HHB 67	HHB 68	HHB 94	HHB 117	HHB 146
		M1	M2	M3	M4	M5	M6	M7	F1	F2	F3	F4	F5	F6	F7	H1	H2	H3	H4	H5	H6	H7
1	0.5	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+	+	-	+	+	-	-
2	0.52	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+
3	0.55	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	0.61	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
5	0.64	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+

+, Present of band; -, absent of band. **Note:** 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*]. H1- Hybrid (HHB50), F1- MS 81A(Female parent of H1), M1- H 90/4-5 (Male parent of H1).

Table 3. Schematic zymogram of POX isozyme in 21 genotypes of pearl millet.

Band	Rf/Rm	H 90/4-5	H 77/833-2	H 77/833-2	H 77/833-2	G 73-107	H 77/29-2	HTP 94/54	MS 81A	MS 81A	MS 843A	MS 842A	ICMA 89111A	HMS 7A	ICMA 95222A	HHB 50	HHB 60	HHB 67	HHB 68	HHB 94	HHB 117	HHB 146
		M1	M2	M3	M4	M5	M6	M7	F1	F2	F3	F4	F5	F6	F7	H1	H2	H3	H4	H5	H6	H7
1	0.5	-	-	-	-	-	-	-	+	+	-	++++	-	+	-	+	++	-	++++	++++	-	-
2	0.52	+++	++++	++++	++++	++++	++++	++	++++	++++	+++	++++	++++	-	+++	++++	++	+++	++++	-	++++	+++
3	0.55	+++	++	++	++	++++	++++	++	++++	++++	++	+++	++++	++++	++++	++++	++	++	+++	++++	++++	++++
4	0.61	+	+++	+++	+++	+++	-	++	++	++	++	-	++++	-	++++	++	++	++	+++	++++	+++	++++
5	0.64	+	++	++	++	++	++	++	++	++	++	-	+++	++++	++	++	++	++	+++	+++	++++	+

+, Very light; ++, light; +++, medium; ++++, dark; -, absent of bands.

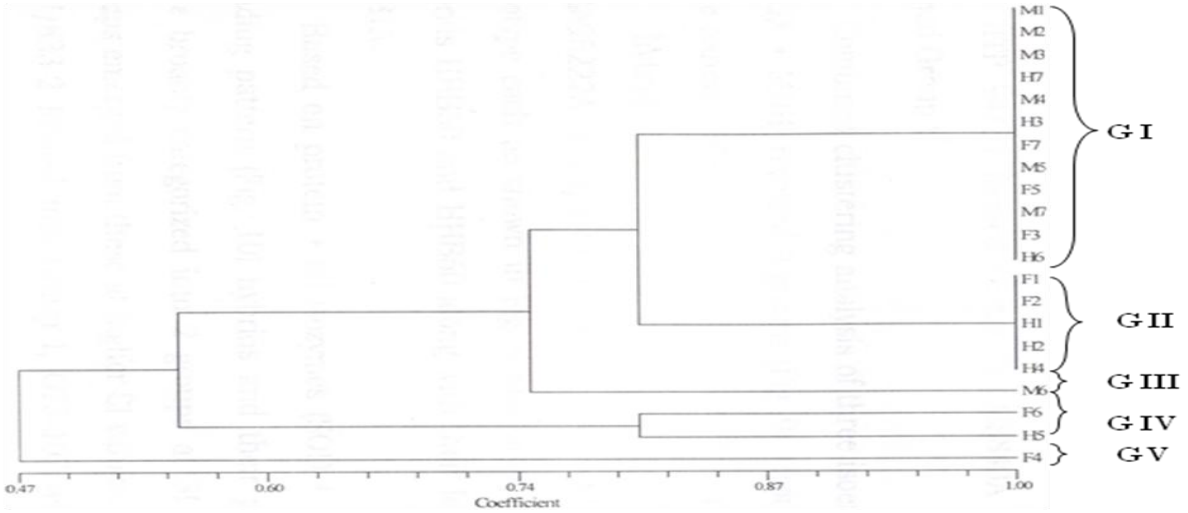

Figure 1. Dendrogram of 21 genotypes of Pearl millet based on POX banding pattern. H1-HHB50, F1-MS 81A, M1-H90/4-5; H2-HHB60, F2- MS81A, M2- H77/833-2; H3-HHB67, F3- MS843A, M3- H77/833-2; H4-HHB68, F4- MS842A, M4- H77 /833-2; H5-HHB94, F5- ICMA89111A, M5- G73-107; H6-HHB117, F6- HMS7 A, M6- H77/29-2; H7-HHB146, F7- ICMA95222A, M7- HTP94/54.

Clustering

All the experimental materials could be grouped into as much as five clusters based on less than 50% Jaccard's similarity coefficient. MS 842A(F4) was only component of a distinct cluster. HHB94(H5) and HMS 7A(F6) of one group; H77/29-2(M6) alone formed another group, MS81A(F1/F2), HHB50 (H1), HHB 60(H2), HHB 68(H4) of another group and H90/4-5(M1), H77/833-2(M2/M3/M4), HHB 146(H7), HHB 67(H3), ICMA 89111A(F7), G73-107(M5), ICMA 89111A(F5), HTP94/54(M7), MS 843A(F3), HHB 117(H6) were the four group of four different distinct clusters (Figure 1). It was also found that

Table 4. Similarity indices between hybrid and their parents.

Hybrid	Female parent	Male parent
HHB 50(H1)	1.0000 MS 81A(F1)	0.8000 H 90/4-5 (M1)
HHB 60(H2)	1.0000 MS 81A(F2)	0.8000 H 77/833-2 (M2)
HHB 67(H3)	0.6000 MS 843A(F3)	1.0000 H 77/833-2 (M3)
HHB 68(H4)	0.6000 MS 842A(F4)	0.8000 H 77/833-2 (M4)
HHB 94(H5)	0.4000 ICMA 89111A(F5)	0.6000 G73-107 (M5)
HHB 117(H6)	0.4000 HMS 7A(F6)	0.8000 H77/29-2 (M6)
HHB 146(H7)	1.0000 ICMA 95222A(F7)	1.0000 HTP94/54(M7)

in each cluster, except the cluster having MS843A (F4) as sole member, the members were 100% similar to each other. Besides, the similarity coefficient ranged from 0.4000 to 0.8000 in all other combinations. Similarity indices based on POX banding pattern (Table 4) revealed that hybrid HHB 50(H1), HHB60 (H2), HHB 67(H3)and HHB 146(H7) completely resembled (SI 1.000) with their female parents MS 81A(F1/F2), MS 843A(F3) and ICMA 95222A(F7). Hybrids HHB 68(H4) and HHB 117(H6) closely resembled (SI 0.8000) towards their male parent than female parents.

The genotypic variation in respect of band numbers indicated differences among the genotypes. Similar type of POX polymorphism among some hexaploid wheat was observed by Gupta et al. (2008). This finding also corroborates the previous findings of Pushpam and Rangasamy (2006), Khandelwal et al. (2004) in rice, Manjunatha et al. (2003) in sugarcane, Philomina and Surendran (2003) in neem, Abideen and Vijayakumar (2002) in *Acasia* species and Roy et al. (2001) in grass pea. Commonness in band numbers as well as Rm values found in the present experiment are indicative of their genetic closeness, whereas band number and their relative mobility values when found different in three genotypes, indicated their genetic distinctness in the molecular level. Difference in band intensity as well as band width is indicative of differences in POX activities. POX activity is increased in plant tissues as defensive response to water stress (Badiani et al., 1990). As there is a significant correlation between root characteristics and water stress condition, the information gathered from POX diversity may help in breeding of Pearl millet varieties with higher POX activity. This can identify cultivar variation which can be used for identifying diverse lines for use as parents in further studies. They can also be used towards a better understanding of phylogentic relationships of different genotypes. However, it is necessary to use molecular markers like random-amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs) and expressed sequence tags (ESTs) to map the entire Pearl millet genome, which could be used to generate novel cultivars through marker-assisted selection, map based cloning and transgenic works.

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