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CHARACTERIZATION OF PEARL MILLET [*PENNISETUM GLAUCUM* (L.) R. BR.] GENOTYPES BY USING SUPEROXIDE DISMUTASE (SOD) ISOZYME

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ABSTRACT

Isozymes are the product of the gene expression and it is a useful basic analytical tool to study the molecular characterization. As an important genetic marker, it is widely used to detect the inheritance and variance among different species or different cultivars within the species at the molecular level. Superoxide dismutase (SOD) is one enzyme of the antioxidase system and generally exists in all kinds of plants. SODs are ubiquitous metalloenzymes that catalyze dismutation of superoxide radicals and prevents organisms from oxidative damage of too much oxygen free radicals, especially the damage to cytoplasm membrane. SOD isozyme had been used for characterization of number of plant species. In Pearl millet SOD isozyme had been extensively used for characterization. The present study conducted to characterize 21 genotypes (seven hybrids, six female parents, five male parents and three Open Pollinated Varieties(OPVs) of pearl millet by using SOD isozyme. Five bands were found ranges from Rm values of 0.44 to 0.91. Bands with Rm values of 0.44, 0.56 and 0.89 were monomorphic for all genotypes. Band with Rm value of 0.48 and 0.91 were found to be polymorphic. All genotypes could be grouped into as much as four clusters based on less than 50% Jaccard's similarity coefficients. Hybrids HHB50 (H1), HHB 67(H3) and HHB 94(H5) were closely resembled (SI= 0.8000) with their female parents *viz,* MS 81A (F1), MS 843A (F3) and ICMA 89111A (F5).

Key words: Characterization, Cluster, Superoxide dismutase.

INTRODUCTION

Pearl millet (*Pennisetum glaucum*) is a nutritious cereal grown on about 10 million ha in India, which is the largest producer of this crop in the world. It ranks third after wheat (*Triticum aestivum*) and rice (*Oryza sativa*) in area in India. It is basically cultivated as a rain fed crop, largely under marginal environment and with no or little external inputs. More than 70 genotypes, of which about 60 are hybrids, have been released in the last 35 years and their number will increase in future due to the ever-changing breeding objectives to meet the current and future demands of the producers and consumers. Apart from this, the Government of India under the obligation of the TRIPS agreement has recently passed the Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPVFRA, 2001) to encourage public/private investment in research and development of new plant varieties by giving protection to the new plant genotypes against unauthorized multiplication of seeds or propagating materials for a specified period. The purpose of the Act will be achieved only when the new varieties are given proper protection under it. The new pearl millet genotypes will be protected under the PPV&FR Act after confirming the distinctness, uniformity and stability (DUS) of new genotypes through DUS testing (PPVFRA, 2001). The DUS of the new genotype will be established by growing the new and existing varieties side by side and comparing them with respect to a set of morphological characters throughout the plant growth period. This will be time-consuming and expensive, requiring large areas of land and skilled personnel often making subjective decisions. Added to this, many of the characters used are quantitative and their expression is altered by environmental factors, which essentially require replication of observations. It also demands costly

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cold storage facilities to store, and seeds of all protected and extant varieties. Further, the number of characters may not be sufficient for discrimination of all the extant and new varieties. There are thus compelling reasons to find more rapid and cost-effective procedures to augment this approach. Biochemical markers assay variation in the polypeptides of proteins and isozymes based on size and/or charge differences. Such differences remain unaffected across different seasons, locations and agronomic practices. Varietal identification and differentiation therefore become more reproducible and objective. The characteristics of a new variety based on electrophoretic patterns of proteins/ isozymes can therefore be compared with those commonly known in any part of the world for establishing its uniqueness. The working group on Biochemical and Molecular Techniques (BMT) of the International Union for the Protection of New Varieties of Plants (UPOV) has in fact identified isozymes markers that could be used as complementary characteristics in maize and soybean DUS testing (UPOV, 1998 and UPOV, 1999) and protein markers in wheat and barley (UPOV, 1996 and UPOV, 1994). However, it is necessary that prior to the use of such markers in DUS testing, it would be essential to evaluate the uniformity and stability of the markers as used for distinct distinctness. Isozymes are the product of the gene expression and it is a useful basic analytical tool to study the molecular characterization. As an important genetic marker, it is widely used to detect the inheritance and variance among different species or different cultivars within the species at the molecular level. Superoxide dismutase (SOD) is one enzyme of the antioxidase system and generally exists in all kinds of plants. SODs are ubiquitous metalloenzymes that catalyze dismutation of superoxide radicals and prevents organisms from oxidative damage of too much oxygen free radicals, especially the damage to cytoplasm membrane. SOD had been used for characterization of number of Plant species (Suso and Moreno, 1986; Mancini *et al.*, 1989; Torres *et al.*, 1995 and Chevreau *et al.*, 1999). In Pearl millet Superoxide dismutase (SOD) isozyme had been extensively used for characterization (Chabbra *et al.*, 1996 and Gulati, 1998). The present study aims to characterize 21 genotypes of Pearl millet by using Superoxide dismutase (SOD) isozyme.

MATERIALS AND METHODS

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 *vi* 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 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 Superoxide dismutase the method followed was that of Wendel and Weeden(1989). The Superoxide dismutase displayed as (anodal bands) white bands in the dark background of gel. 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 ranges from Rm values of 0.44 to 0.91(Table - 2). Bands with Rm Values of 0.44, 0.56 and 0.89 were monomorphic for all genotypes. Band with Rm value of 0.48 and 0.91 were found to be polymorphic.

Bands with Rm value of 0.48 (Table - 3) were found in dark intensity at HHB 50(H1), HHB 60(H2), HHB 67(H3) and MS81A (F1/F2) and HHB 68(H4), medium intensity for MS 842A (F4) and H77/29-2(M6). ICMA 89111A (F5) distinctly had shown light intensity band. Bands with Rm value of 0.91 were showed light intensity for HHB 94(H5), HHB 117(H6), HHB 146(H7), MS843A

TABLE 1: List of pearl millet genotypes and their pedigree.

Genotype	Status	Pedigree	Year of Release	Origin
HHB 50	Hybrid	MS 81A × H 90/4-5	1987	CCS HAU, Hisar
HHB 60	Hybrid	MS 81A × H 77/833-2	1988	CCS HAU, Hisar
HHB 67	Hybrid	MS 843A × H 77/833-2	1990	CCS HAU, Hisar
HHB 68	Hybrid	MS 842A × H 77/833-2	1993	CCS HAU, Hisar
HHB 94	Hybrid	ICMA 89111A × G 73-107	1999	CCS HAU, Hisar
HHB 117	Hybrid	HMS 7A × H 77/29-2	2002	CCS HAU, Hisar
HHB 146	Hybrid	ICMA 95222A × HTP 94/54	2002	CCS HAU, Hisar
MS 81A	CMS	Derived from Tift $23D_2$ after irradiation	1981	ICRISAT, Hyderabad
MS 843A	CMS	Selected from AKM 2068 for Downy mildew resistance	1984	ICRISAT, Hyderabad
MS 842A	CMS	Re Selected from AKM 2068 for Downy mildew resistance	1984	ICRISAT, Hyderabad
ICMA 89111A	CMS	881A cytoplasm source(B_1) backcrossed to ICMB 89111	1989	ICRISAT, Hyderabad
HMS 7A	CMS	Developed by backcrossing from the cross 81 A × 35(81B × 69B)	1991	ICRISAT, Hyderabad
ICMA 95222A	CMS	81A cytoplasm(A_1) source back crossed to ICMB 95222	1995	ICRISAT, Hyderabad
H 90/4-5	Restorer	Developed by selecting selfed progenies form synthetic HSI	1976	CCS HAU, Hisar
H 77/833-2	Restorer	Developed by selfing a Haryana land race population	1976	CCS HAU, Hisar
G 73-107	Restorer	Developed by selecting selfed progenies of GAM 73	1976	CCS HAU, Hisar
H 77/29-2	Restorer	Developed by selecting selfed plants from Rajasthan landrace	1976	CCS HAU, Hisar
HTP 94/54	Restorer	Developed by selecting selfed progenies of high tillering of Tago population	1992	CCS HAU, Hisar
HC 4	OPV	Developed by intermating seven inbred lines	1985	CCS HAU, Hisar
HC 10	OPV	Bred by random mating 15 S1 progenies of NELC population	1999	CCS HAU, Hisar
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 Superoxide Dismutase (SOD) isozyme in 21 genotypes of pearl millet.

Bands	Rf/Rm	H 90/4-5 M1	H77/833-2 M2	H77/833-2 M3	H77/833-2 M4	G 73-107 M5	H77/29-2 M6	HTP 4/54 M7	MS 81A F1	MS 81A F2	MS 843A F3	MS 842A F4	ICMA 89111A F5	HMS 7A F6	ICMA 95222A F7	HHB50 H1	HHB 60 H2	HHB 67 H3	HHB 68 H4	HHB 94 H5	HHB 117 H6	HHB146 H7
1	0.44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	0.48	-	-	-	-	-	+	-	+	+	-	+	+	-	-	+	+	+	+	_	-	-
3	0.56	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	0.89	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	0.91	+	-	-	-	-	-	-	+	+	+	-	+	+	+	-	+	+	-	+	+	+

\+ Presence of band, - Absence of band

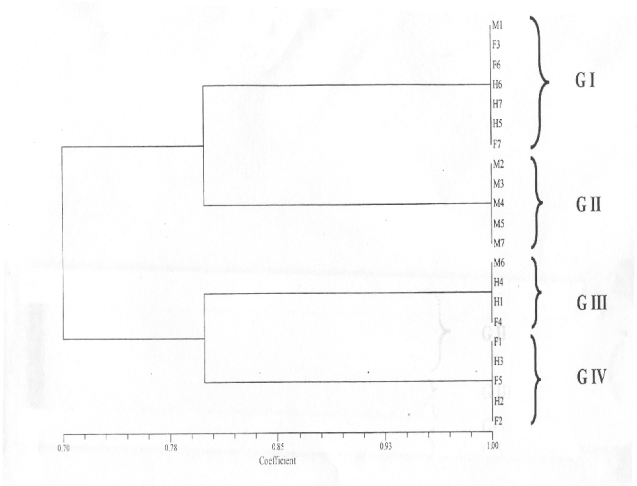
Note:

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)

(F3), MS 842A (F4), HMS 7A (F6), and medium intensity for H90/4-5(M1), HHB 67(H3) and dark intensity for HHB 60(H2), MS 81A (F1/F2) and ICMA 95222A (F7).

Bands with Rm value 0.44 were present in all genotypes, in that HHB 68 (H4), HHB 94(H5), MS 843A (F3), MS 842A (F4), H 77/833-2(M2/M3/M4) and G 73-107 (M5) were of medium intensity and all other genotypes showed dark intensity bands. H 90/4-5(M1), MS 843A (F3) and HHB 68(H4) showed medium intensity bands at Rm value of 0.56 and rest of the genotypes showed dark intensity bands. H 77/833-2 (M2/M3/M4), HMS 7A(F6) and HHB 148(H7) were showed medium intensity bands at Rm value 0.89 and all other genotypes showed dark intensity bands.

Clustering: The 21 genotypes grouped into four clusters (Fig.1) based on less than 50% Jaccard's similarity coefficients. HHB 94 (H5), HHB117 (H6), HHB146 (H7), MS 843A (F3), HMS 7A (F6), ICMA 95222A (F7) and H 90/4-5(M1) were formed one cluster. Second cluster consisted of H 77/833-2 (M2/M3/M4), G73-107(M5) and HTP 94/54 (M7). HHB 50(H1), HHB 68(H4), MS 842A (F4) and H 77/29-2 (M6) were formed third cluster. Fourth cluster consisted of HHB 60(H2), HHB 67(H3), MS 81A (F1/F2) and ICMA 89111A (F5).



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

FIG. 1: Dendrogram of 21 genotypes of Pearl millet based on banding pattern Superoxide Dismutase (SOD) isozyme

It was found that in each cluster the genotypes were 100% similar. Besides the similarity coefficients ranged from 0.6000 to 0.8000(Table - 4). Hybrids HHB 60(H2), HHB 68(H4), HHB 117(H6) and HHB 147(H7) resembled completely (SI= 1.0000) (Table -5) with their female parents *viz.*, MS 81A (F2), MS 842A (F4), HMS 7A(F6) and ICMA 95222A(F7). While, hybrids HHB50 (H1), HHB 67(H3) and HHB 94(H5) resembled closely (SI= 0.8000) with their female parents *viz.*, MS 81A (F1), MS 843A (F3) and ICMA 89111A (F5). In the present study, polymorphism for isozyme was observed in the anodal migrating zone of the gel, indicating the suitability of isozymes in establishing the varietal distinctness. Similar reports have been reported by earlier workers in case of Pearl millet germplasm (Tostain *et al.*, 1987; Varier *et al.*, 1990; Varier *et al.*, 1993; Varier *et al.*, 1995; Rao *et al.*, 2007; Kumar *et al.*, 2004 and Kumar *et al.*, 2007).

Variations within cultivars for electrophoretic profiles of different markers have also been reported in other crops (Hayward and McAdam, 1977; Almgard and Landegren, 1974; Almagard and Clapham, 1975; Draper and Craig, 1981 and May *et al.*, 1982). This was attributed to breeding methods, where selections were based on morphological characteristics and not on electrophoretic markers. Hence, residual heterozygosity is likely to be left at the respective loci of these markers, leading to intravarietal segregation in subsequent generations. Thus there is always a chance for the new or purified genotype being non-uniform with respect to isozymes, even though it is uniform with respect to other plant morphological characters. UPOV, therefore, had appointed a special committee called the 'Biochemical and Molecular Technique Group' to work on the possibilities of using biochemical and molecular markers in DUS testing (Sing *et al.*, 2004). One such possibility could be allowing maximum limits for variants based on the genetics of isozyme markers, while evaluating the genotype for its uniformity. Utmost care has to be taken while giving protection to the new variety based on its DUS established using biochemical markers. Otherwise, it may lead to problems of piracy during later stages of variety

TABLE 3: Schematic Zymogram of Superoxide Dismutase (SOD) isozyme in 21 genotypes of pearl millet.

		H 90/4-5	H77/833-2	H77/833-2	H77/833-2	G 73-107	H77/29-2	HTP 94/54	MS 81A	MS 81A	MS 843A	MS 842A	ICMA 89111A	HMS 7A	ICMA 95222A	HHB50	HHB 60	HHB 67	HHB 68	HHB 94	HHB 117	HHB146
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	044	D	M	M	M	M	D	D	D	D	M	M	D	D	D	D	D	D	M	M	D	D
2	048	-	-	-	-	-	M	-	D	D	-	M	vL	-	-	D	D	D	M	_	-	-
3	056	M	D	D	D	D	D	D	D	D	M	D	D	D	D	D	D	D	M	D	D	D
4	089	D	M	M	M	D	D	D	D	D	D	D	D	M	D	D	D	D	D	D	D	M
5	091	M	-	-	-	-	-	-	D	D	VL	VL	VL	VL	D	-	D	M	-	VL	VL	VL

D- Dark, M- Medium, L-Light, VL-Very light, - Absent of bands

TABLE 4: Similarity Indices among 21 genotypes of pearl millet based on Superoxide Dismutase (SOD) isozyme.

GENOTYPES	H 90/4-5	H77/833-2	H77/833-2	H77/833-2	G 73-107	H77/29-2	HTP 94/54	MS 81A	MS 81A	MS 843A	MS 842A	ICMA 89111A	HMS 7A	ICMA 95222A	HHB50	HHB 60	HHB 67	HHB 68	HHB 94	HHB 117	HHB146
	M1	M2	M3	M4	M5	M6	M7	F1	F2	F3	F4	F5	F6	F7	H1	H2	H3	H4	H5	H6	H7
M1	1.0000																				
M2	0.8000	1.0000																			
M3	0.8000	1.0000	1.0000																		
M4	0.8000	1.0000	1.0000	1.0000																	
M5	0.8000	1.0000	1.0000	1.0000	1.0000																
M6	0.6000	0.8000	0.8000	0.8000	0.8000	1.0000															
M7	0.8000	1.0000	1.0000	1.0000	1.0000	0.8000	1.0000														
F1	0.8000	0.6000	0.6000	0.6000	0.6000	0.8000	0.6000	1.0000													
F2	0.8000	0.6000	0.6000	0.6000	0.6000	0.8000	0.6000	1.0000	1.0000												
F3	1.0000	0.8000	0.8000	0.8000	0.8000	0.6000	0.8000	0.8000	0.8000	1.0000											
F4	0.6000	0.8000	0.8000	0.8000	0.8000	1.0000	0.8000	0.8000	0.8000	0.6000	1.0000										
F5	0.8000	0.6000	0.6000	0.6000	0.6000	0.8000	0.6000	1.0000	1.0000	0.8000	0.8000	1.0000									
F6	1.0000	0.8000	0.8000	0.8000	0.8000	0.6000	0.8000	0.8000	0.8000	1.0000	0.6000	0.8000	1.0000								
F7	1.0000	0.8000	0.8000	0.8000	0.8000	0.6000	0.8000	0.8000	0.8000	1.0000	0.6000	0.8000	1.0000	1.0000							
H1	0.6000	0.8000	0.8000	0.8000	0.8000	1.0000	0.8000	0.8000	0.8000	0.6000	1.0000	0.8000	0.6000	0.6000	1.0000						
H2	0.8000	0.6000	0.6000	0.6000	0.6000	0.6000	0.8000	1.0000	1.0000	0.8000	0.8000	1.0000	0.8000	0.8000	0.8000	1.0000					
H3	0.8000	0.6000	0.6000	0.6000	0.6000	0.6000	0.8000	1.0000	1.0000	0.8000	0.8000	1.0000	0.8000	0.8000	1.0000	1.0000	1.0000				
H4	0.6000	0.8000	0.8000	0.8000	0.8000	1.0000	0.8000	0.8000	0.8000	0.6000	1.0000	0.8000	0.6000	0.6000	1.0000	0.8000	0.8000	1.0000			
H5	1.0000	0.8000	0.8000	0.8000	0.8000	0.6000	0.8000	0.8000	0.8000	1.0000	0.6000	0.8000	1.0000	1.0000	0.6000	0.8000	0.8000	0.6000	1.0000		
H6	1.0000	0.8000	0.8000	0.8000	0.8000	0.6000	0.8000	0.8000	0.8000	1.0000	0.6000	0.8000	1.0000	1.0000	0.6000	0.8000	0.8000	0.6000	1.0000	1.0000	
H7	1.0000	0.8000	0.8000	0.8000	0.8000	0.6000	0.8000	0.8000	0.8000	1.0000	0.6000	0.8000	1.0000	1.0000	0.6000	0.8000	0.8000	0.6000	1.0000	1.0000	1.0000

TABLE 5: Similarity indices between hybrids and their parents.

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

protection. Hence if biochemical markers are used for granting varietal protection, then the breeders should see that their variety is uniform with respect to the associated biochemical marker, or they should claim the protection stating the proportion of each variant of their variety with respect to a particular biochemical marker, or only such biochemical markers should be used for granting varietal registration that are associated with any of the morphological characters. Thus while achieving the uniformity of the associated marker, the uniformity of the respective biochemical marker will be achieved automatically.

CONCLUSION

From the present study it has been found that the isozyme markers are highly polymorphic among pearl millet cultivars, stable over generations, unaffected by environment and not associated with any morphological characters. These attributes which have been tested and confirmed, call for its consideration as an ideal additional descriptor for establishing distinctness and stability of new pearl millet cultivars, which in turn serve the purpose of granting plant variety protection. However, before using this descriptor in DUS testing, its validity for testing the uniformity of genotypes has to be reconfirmed in other Pearl millet.

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