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RESEARCH PAPER

Genetic purity and diversity in isoenzyme *Peroxidase* of pearl millet hybrids [*Pennisetum glaucum* (L.) Br. R.]

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The prospective of Peroxidase Isozyme biochemical marker, have been investigation in the present study, ten pearl millet hybrids [*Pennisetum glaucum* (L.) R. Br.] Genotypes were used to examine the suitability of peroxidase enzymes for characterization of pearl millet genotypes. Band number 1 (Rm = 0.120) was present in three hybrids *i.e.* GHB-526, GHB-538 and GHB-719. Band number 2 (0.158) was also present in three hybrids *i.e.* GHB-538, GHB-577 and GHB-719. Band number 3 (Rm = 0.380) was present in only one hybrid *i.e.* GHB-719 while band number 4 (Rm = 0.429) was present in five hybrids GHB-538, GHB-719, GHB-732, GHB-235 and GHB-757. All the seven bands were present in only one hybrid GHB-719. 9 DAG stage was found to be more effective as compared to 3 DAG and 6 DAG by showing more number of bands. Among the 10 genotypes studied, two could be differentiated from each other Total 13 bands of peroxidase isozymes were observed at 3, 6 and 9 day after germination (DAG). A total of 15 alleles were generated by isozymes at different DAG (3, 6 and 9 days) with an average of 5 bands per day. Banding pattern at 6 DAG showed that band number1, 2 and 3 (Rm = 0.525, 0.573 and 0.600) were present in all the hybrids except for GHB-744, in band number 1. At 9 DAG seven bands of peroxidase isozymes were observed having Rm value of 0.120, 0.158, 0.380, 0.429, 0.430, 0.478, and 0.760.

Key words : Genetic purity, Pearl millet hybrids, ADH, GOT

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INTRODUCTION

Selection criteria based on varietal identification is very narrow, genetic base of pearl millet germplasm used by the breeders, morphological markers are of limited utility in characterization of the genotypes and the environmental effects on the expression of these characters and the time required for obtaining results are time consuming therefore the time available for ensuring the genetic purity of seed lots produced in season end is very short. So there is need for identification of a descriptor that is simple to use, rapid, reproducible and not influenced significantly by environment). Hence, the present investigation was carried out to detect the polymorphism among ten pearl millet hybrids. In pearl millet, among the different isozymes (*viz.*,ADH, peroxidases, GOT and MDH), esterases were extensively studied because of the good resolution obtained and the wide variability available in the entire *Pennisetum* gene pool as compared to other enzymes (Lagudah and Hanna, 1989). However the polymorphisms of Peroxidase marker between the pearl millet genotypes have to be established before using it as descriptor. In

light of this preliminary information, the study was conducted to characterize the pearl millet hybrids using peroxidase marker. The term isozyme proposed as multiple molecular forms of an enzyme, sharing a catalytic activity derived from the tissue (Markert and Moller, 1959) but differ in their physical properties. Electrophoretic pattern of soluble enzymes represent direct manifestation. The difference in the isozyme banding pattern is due to variations in the amino acid content of the molecule, which in turn is dependent on the sequence of nucleotides in the DNA (Micales *et al.*, 1986). Hence, the present investigation was carried out to detect the polymorphism among ten pearl millet hybrids were tested for peroxidase isozyme profiling at three different seedlings stages *viz.,* 3, 6 and 9 day after germination. 9 DAG stage was found to be more effective as compared to 3 DAG and 6 DAG, as pattern of isozymes was distinct using isozyme analysis.

RESEARCH METHODOLOGY

The hybrids used for the present study were supplied by the Research Scientist, Millet Research Station, Junagadh Agricultural University, Jamnagar *viz.,* GHB-235, GHB-316 GHB-526, GHB-538, GHB-558,GHB-577, GHB-719, GHB-732 ,GHB-744 ,GHB-757. The present investigation on evaluation for Genetic Purity and Diversity in Isoenzyme estarase of ten Pearl Millet Hybrids [*Pennisetum glaucum* (L.)Br. R.] was carried out at the Department of Biochemistry and Biotechnology, Junagadh Agricultural University (JAU), Junagadh. Genetically pure seeds of pearl millet hybrids were germinated in petri dishes containing filter paper and distilled water. The seeds were kept at 20^{0}C for 9 days. The seedlings were taken during following intervals 3rd, 6th and 9th days after germination for Isoenzyme study to analyze the location and seasonal effects on the expression of esterase marker.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Peroxidases:

Peroxidase extracted from tissues was analyzed. The sample material was homogenized in 5-fold volume of 10 mM sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 rpm for 10 min and the supernatant was used as enzyme source. All operations were carried out at 0–4^{0}C. Native PAGE of the sample extracts was carried out. Native PAGE of the sample extracts was carried out. The gel was incubated in the O-dianisidine, 0.2 g, Acetic acid,10 ml, Methanol,40 ml and volume make up to100 ml. Hydrogen peroxide (3%) was carefully added drop by drop in the gel up to the appeance of bright blue bands. When the bands were stained sufficiently, the reaction was arrested by immersing the gel in 7 per cent acetic acid solution for 10 min (Van Loon, 1971). Each electrophoretic analysis was replicated. The relative mobility (Rm) of each band was measured in each zymogram for every hybrid tested using the following equation (Eeswara and Peiris, 2001). Rm=Distance migrated by the enzyme band/ Distance migrated by the dye marker. All gel photographs were scored for the presence/absence for Isozymes and Protein marker bands. For statistical analysis, data were recorded

Table 1 : Rm values of banding pattern of peroxidase isozyme from pearl millet seedling at 3, 6 and 9th day after germination

Hybrids/Band Number (Rm values)	3 DAG					6 DAG			9 DAG						
	1	2	3	4	5	1	2	3	1	2	3	4	5	6	7
	0.066	0.131	0.377	0.792	0.836	0.525	0.573	0.6	0.12	0.158	0.38	0.429	0.43	0.478	0.76
GHB-526	1	1	0	0	1	1	1	1	1	0	0	0	1	1	1
GHB-538	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1
GHB-558	1	1	0	1	1	1	1	1	0	0	0	0	1	1	1
GHB-577	1	1	0	1	1	1	1	1	0	1	0	0	1	1	1
GHB-719	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
GHB-732	1	1	0	1	0	1	1	1	0	0	0	1	1	1	1
GHB-744	1	1	0	0	0	0	1	1	0	0	0	0	1	1	1
GHB-757	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1
GHB-235	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1
GHB-316	1	1	0	1	0	1	1	1	0	0	0	0	1	1	1

Table 2 : Parameters used for esterase isozyme study of pearl millet hybrids

Isozymes	Day after germination (DAG)	Total no. of allele (A)	Polymorphic band (B)	Polymorphic % (B/A)	PIC value
Peroxidase	3	5	5	100	0.757
Peroxidase	6	3	1	33.33	0.665
Peroxidase	9	7	4	57.14	0.804
Mean		5	3.33	63-49	0.742

for the presence/ absence of bands, that were clearly resolved on the gel were scored as 1 for presence 0 for absence, respectively across all the 10 hybrids of pearl millet. The dendrogram and genetic similarity values based on genetic distance method using UPGMA by NTSYS pc version 2.02i.(Rohlf,1998)

Isozymes pattern of pearl millet hybrids :

The results revealed that At 3 DAG five bands of peroxidase isozymes were observed having Rm value of 0.066, 0.131, 0.377, 0.792 and 0.836. Banding pattern at 3 DAG (Fig. 1 and Table 1) showed that band number 1 and 2 (Rm = 0.066 and 0.131) were present in all the hybrids. Band number 3 (Rm = 0.377) was present only in GHB-719. Band number 4 (Rm = 0.792) was present in seven hybrids i.e. GHB-558, GHB-577, GHB-719, GHB-732, GHB-316, GHB-235 and GHB-757. Band number 5 (Rm = 0.836) was present in all hybrids except for three hybrids *i.e.* GHB-732, GHB-744 and GHB-316. At 6 DAG three bands of peroxidase isozymes were observed having Rm value of 0.525, 0.573 and 0.600. Banding pattern at 6 DAG (Fig. 1 and Table 2) showed that band number1, 2 and 3 (Rm = 0.525, 0.573 and 0.600) were present in all the hybrids except for GHB-744, in band number 1. At 9 DAG seven bands of peroxidase isozymes were observed having Rm value of 0.120, 0.158, 0.380, 0.429, 0.430, 0.478, and 0.760. Banding pattern at 9 DAG (Fig. 2) showed that band number 5, 6 and 7 (Rm = 0.430, 0.478, and 0.760) were present in all the hybrids. Band number 1 (Rm = 0.120) was present in three hybrids *i.e.* GHB-526, GHB-538 and GHB-719. Band number 2 (0.158) was also present in three hybrids *i.e.* GHB-538, GHB-577 and GHB-719. Band number 3 (Rm = 0.380) was present in only one hybrid *i.e.* GHB-719 while band number 4 (Rm = 0.429) was present in five hybrids GHB-538, GHB-719, GHB-732, GHB-235 and GHB-757. All the seven bands were present in only one hybrid GHB-719. 9 DAG stage was found to be more effective as compared to 3 DAG and 6 DAG by showing more number of bands.

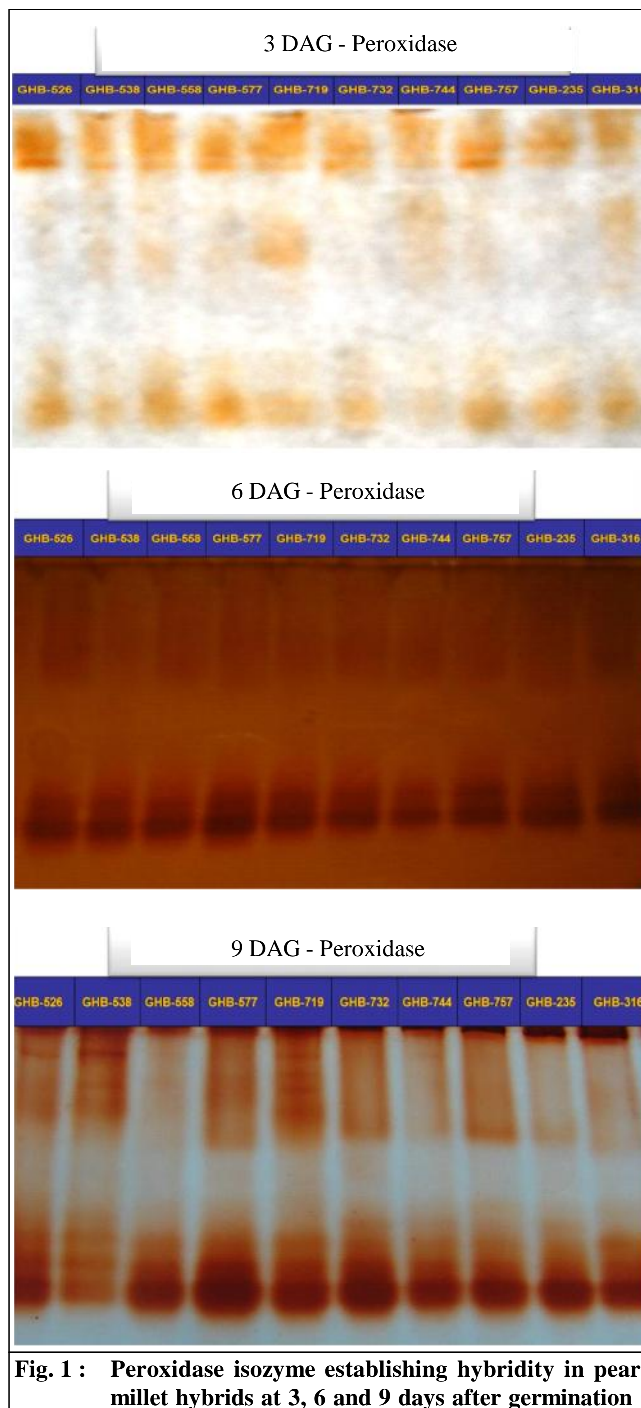

Fig. 1 : Peroxidase isozyme establishing hybridity in pearl millet hybrids at 3, 6 and 9 days after germination

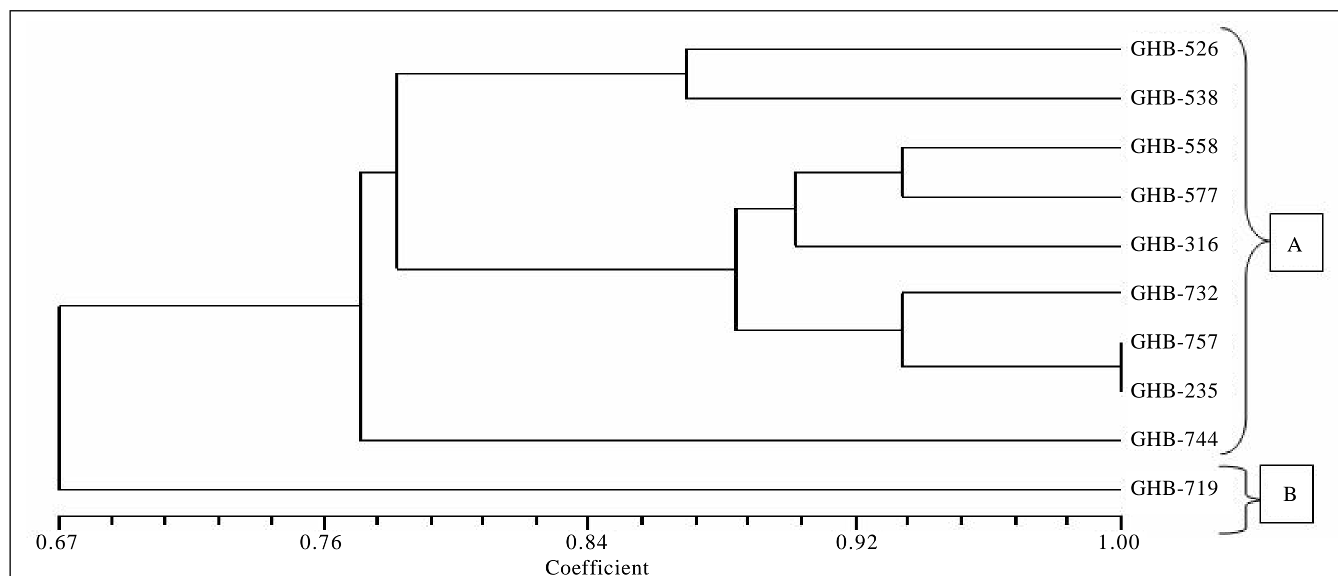

Fig. 2 : Dendrogram depicting the genetic relationship among ten pearl millet hybrids based on three stage isoenzymes (Peroxidase) data

Genetic analysis :

A total of 15 alleles were generated by isozymes at different DAG (3, 6 and 9 days) with an average of 5 bands per day. Only easily resolved and bright isozymes bands were counted. 9 DAG found highest no of band *i.e.* seven. All three days Polymorphic information content (PIC) value was 0.742. Genetic distance was determined for each pair of the ten populations using the methods of Jaccard (1980). Analysis of pairwise genetic distance revealed that the genetic similarity was minimum 0.466 and maximum 1.000. Cluster analysis was carried out by the UPGMA method on the Nei and Lei's (1979) based on genetic distance. The position of the hybrid in different cluster is presented in Fig. 2. The dendogram constructed with UPGMA based genetic distance revealed that ten pearl millet hybrids fell into two main clusters A and B. The cluster A divided in two sub cluster A1 and A2. Sub cluster A1 further divided in two sub-sub cluster A1a and A1b. A1a consist of two hybrids GHB-526 and GHB-538, while A1b also further divided in A1b(I) and A1b(II). A1b(I) comprised three hybrids *i.e.* GHB-558, GHB-577 and GHB-316 and A1b(II) also comprised three hybrids *i.e.* GHB-732, GHB-757 and GHB-235, A2 consist only one hybrid GHB-744. Cluster B included only one hybrids GHB-719. The results revealed that GHB-757 and GHB-235 were having highest similarity while lowest similarity (46%) was found between GHB-719 and GHB-744. GHB-719 showed maximum variability compared to other nine hybrids. Genetic variation was evaluated in 7, 12, 1 and 4 varieties of bread wheat, durum wheat, triticale and barley, respectively, by electrophoretic analysis of 6 enzyme systemviz peroxidases (POX), esterases (EST), glutamate oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP), acid phosphatase (ACP) and endopeptidase (ENP) (Aouad *et al*., 1998).

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