RESEARCH PAPER | Asian Journal of Bio Science, Volume **9** | Issue 1 | April, 2014 | 76-79
Received : 06.03.2014; **Revised :** 15.03.2014; **Accepted :** 23.03.2014

Evaluation for genetic purity and diversity in isoenzyme ~- *Esterases* of ten pearl millet hybrids (*Pennisetum glaucum*)

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The prospective of biochemical marker like seed esterases, was investigated in the present study. Ten pearl millet hybrids (*Pennisetum glaucum*) genotypes were used to examine the suitability of esterases for characterization of pearl millet genotypes. Banding pattern at 9 DAG showed that band number1, 2 and 4 (Rm = 0.332 0.549 and 0.674) were present in all the hybrids. Band number 3 (Rm = 0.636) was present in six hybrids *i.e.* GHB-526, GHB-558, GHB-577, GHB-732, GHB-744 and GHB-757. Band number 5 (Rm = 0.728) was present in all hybrids except for GHB-538. Among the 10 genotypes studied, two could be differentiated from each other. Total 13 bands of esterase isozymes were observed at 3, 6 and 9 day after germination (DAG). Polymorphism was observed 75%, 100 per cent and 40 per cent at 3, 6 and 9 DAG, respectively. Genetic distance revealed that ten pearl millet hybrids fell into two main clusters A and B. The cluster A included two sub clusters A1 and A2. Sub-cluster A1 further divided in two sub-sub cluster A1a and A1b. A1a comprised of four hybrids *i.e.* GHB-526, GHB-558, GHB-577and GHB-235, while A1b consisted of only one hybrid GHB-744. Sub cluster A2 comprised of three hybrids *i.e.* GHB-732, GHB-757 and GHB-316. Cluster B included two hybrids GHB-538 and GHB-719. Thus, results clearly indicated that highest similarity was found between hybrids GHB-732 and GHB-757 while lowest similarity was observed between hybrids GHB-538 and GHB-744.

Key words : Genetic purity, Diversity, Isoenzyme, Pearl millet, Hybrids

How to cite this paper : Vaja, M.B., Rathod, P.J. and Mandavia, M.K. (2014). Evaluation for genetic purity and diversity in isoenzyme ~-*Esterases* of ten pearl millet hybrids. *Asian J. Bio. Sci.*, **9** (1) : 76-79.

INTRODUCTION

Selection criteria based on varietal identification is very narrow. 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. However, the polymorphisms of esterase 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 esterase 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 esterase 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, 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 the intervals of 3rd, 6th and 9th days after germination for isoenzyme study to analyze the location and seasonal effects on the expression of esterase marker.

~-Esterases :

~-Esterases extracted from ten seeds were analyzed by the alkaline PAGE procedure described by Dadlani and Varier (1993). 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. The gel was incubated in a solution given below at 37^0 C for 20-30 min, in dark conditions. For the extraction condition sodium dihydrogen phosphate (2.8 g), disodium hydrogen phosphate (1.1 g), fast blue RR salt (0.2 g), α- naphthyl acetate (0.03 g) was used to make up 200ml. The enzyme reaction was stopped by adding a mixture of methanol: water: acetic acid: ethyl alcohol in the ratio 10:10:2:1 (Desborough and Peloquin, 1967). 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 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).

RESEARCH FINDINGS AND ANALYSIS

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

Isozymes pattern of pearl millet hybrids :

The results revealed that at 3 DAG four bands of estrease isozymes were observed having Rm value of (0.594), (0.659), (0.718) and (0.782). Banding pattern at 3 DAG showed that band number 1 (Rm = 0.594) and 4 (Rm = 0.782) were present in all the hybrids. Band number 2 (Rm = 0.659) was present in six hybrids *i.e.* GHB-538, GHB-558, GHB-719, GHB-577, GHB-744 and GHB-235, while band number 3 (Rm = 0.718) was present only in four hybrids *i.e.* GHB-538, GHB-558, GHB-719 and GHB-577. At 6 DAG, four bands of estrease isozymes were observed having Rm value of 0.277, 0.413, 0.451 and 0.489. Banding pattern at 6 DAG (Fig. 1 and Table 1) showed that band number 1 (Rm = 0.277) was present in eight hybrids. Band number 2 (Rm =0.413) was present in only three hybrids *i.e.* GHB-719, GHB-757 and GHB-744.Band number 3 (Rm =0.451) was present in all hybrids but absent in a GHB-316. Band number 4 (Rm =0.489) was present in all hybrids except

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

Hybrids/Band	3 DAG				6 DAG				9 DAG				
number (Rm	1	2	3	4	1	2	3	4	1	2	3	4	5
values)	(0.594)	(0.659)	(0.718)	(0.782)	(0.277)	(0.413)	(0.451)	(0.489)	(0.332)	(0.549)	(0.636)	(0.674)	(0.728)
GHB-526	1	0	0	1	1	0	1	1	1	1	1	1	1
GHB-538	1	1	1	1	1	0	1	0	1	1	0	1	0
GHB-558	1	1	1	1	1	0	1	1	1	1	1	1	1
GHB-577	1	1	1	1	0	0	1	1	1	1	1	1	1
GHB-719	1	1	1	0	1	1	1	0	1	1	0	1	1
GHB-732	1	0	0	1	1	0	1	1	1	1	1	1	1
GHB-744	1	1	0	1	0	1	1	1	1	1	1	1	1
GHB-757	1	0	0	1	1	1	1	1	1	1	1	1	1
GHB-235	1	1	0	1	1	0	1	1	1	1	0	1	1
GHB-316	1	0	0	1	1	0	0	1	1	1	0	1	1

for two hybrids *i.e.* GHB-538 and GHB-719. At 9 DAG five bands of estrease isozymes were observed having Rm value of 0.332, 0.549, 0.636, 0.674and 0.728. Banding pattern at 9 DAG (Fig. 1 and Table 1) showed that band number 1, 2 and 4 (Rm = 0.332 0.549 and 0.674) were present in all the hybrids. Band number 3 (Rm = 0.636) was present in six hybrids *i.e.* GHB-526, GHB-558, GHB-577, GHB-732, GHB-744 and GHB-757. Band number 5 (Rm = 0.728) was present in all hybrids except for GHB-538.

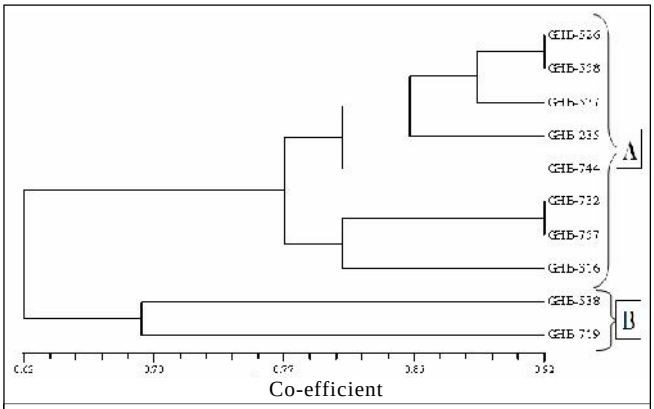

Fig. 1 : Dendrogram depicting the genetic relationship among ten pearl millet hybrids based on three stage isoenzymes estarase data

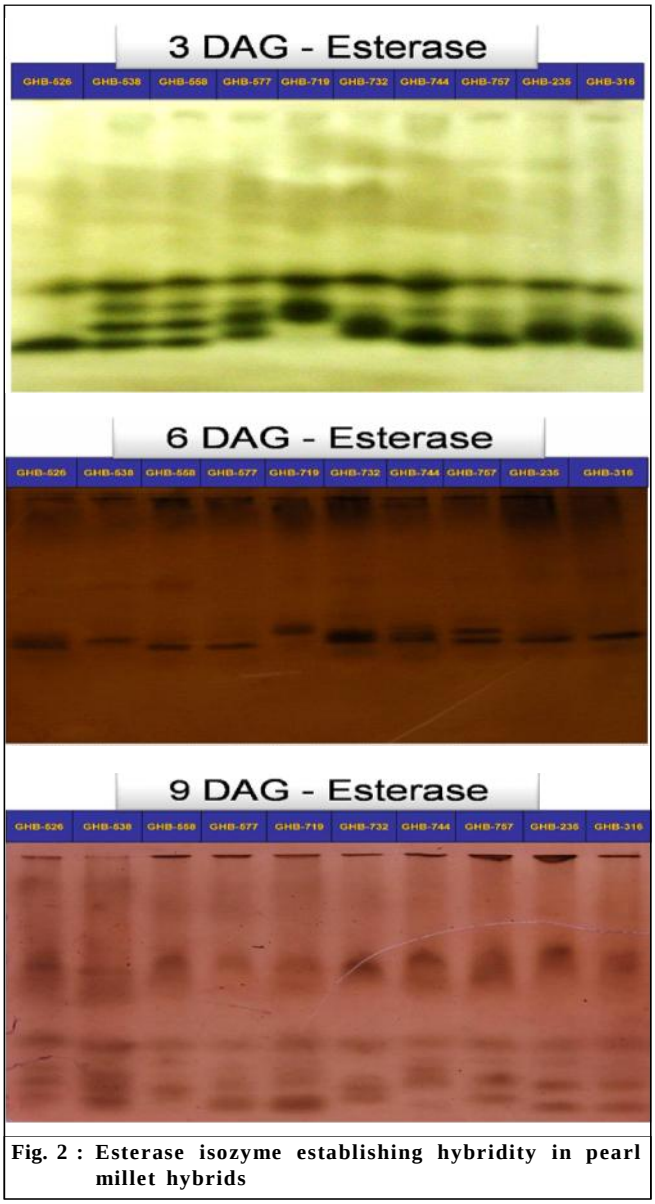

Fig. 2 : Esterase isozyme establishing hybridity in pearl millet hybrids

Genetic analysis :

A total of 13 alleles were generated by isozymes at different DAG (3, 6 and 9 days) with an average of 4.3 bands per day. Only easily resolved and bright isozymes bands were counted. 9 DAG found highest no. of band *i.e.* five. All three stage polymorphic information content (PIC) value 0.745 was found (Table 2). Genetic distance was determined for each pair of the ten populations using the methods of Jaccard (1980) (Table 2). Analysis of pairwise genetic distance revealed that the minimum similarity was 0.461and maximum similarity was 0.923. Cluster analysis was carried out by the UPGMA method 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 included two sub clusters A1 and A2. Sub-cluster A1 further divided in two sub-sub cluster A1a and A1b. A1a comprised of four hybrids *i.e.* GHB-526, GHB-558, GHB-577and GHB-235, while A1b consisted of only one hybrid GHB-744. Sub cluster A2 comprised of three hybrids *i.e.* GHB-732, GHB-757 and GHB-316. Cluster B included two hybrids GHB-538 and GHB-719. Thus, results clearly indicated that highest similarity was found between hybrids GHB-732 and

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
Esterase	3	4	3	75	0.722
Esterase	6	4	4	100	0.721
Esterase	9	5	2	40	0.794
Mean		4.3	3	71.66	0.745

GHB-757 while lowest similarity was observed between hybrids GHB-538 and GHB-744. Kumar *et al.* (2004) studied esterases in 47 pearl millet genotypes comprising 14 hybrids and their parental lines. Among the 47 genotypes studied, 36 could be differentiated from each other and 11 were grouped into four categories. Esterases were also found suitable for testing hybrid purity.

Acknowledgement :

The first author is grateful to the Biotechnology Department and Food Testing laboratory N.A.B.L., accredited, JAU., Junagadh for providing research facilities during the period of this work. We thank the Pearl millet Breeders of GAU – Jamnagar for providing genetically pure seeds of the pearl millet hybrids.

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