

Morphological and Isozyme diversity in the accessions of two cultivated species of barnyard millet

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Abstract: Present study was conducted to access genetic diversity among 33 accessions of two cultivated species of barnyard millet i.e., *Echinochloa crus-galli* (15 accessions) and *E. frumentacea* (18 accessions). Though crop possesses great nutritional value, little attention has been paid for the improvement of this crop. Peroxidase and esterase analysis showed seven loci possessing 23 alleles in all 33 accessions. Esterase was found to be more useful to assess diversity with more polymorphism in comparison to peroxidase. Based on allozyme frequencies, all the accessions of both the species were grouped separately in to two different groups at a linkage distance of 6.2. The data would be important in detailing the level of variation and relationship within and between species to plan future domestication trials and to manage the wild species collection that is available in the gene banks. [Nature and Science 2010;8(7):71-76]. (ISSN: 1545-0740).

Key words: Barnyard millet, genetic diversity, isozyme analysis, peroxidase, esterase

1. Introduction

Barnyard millet (*Echinochloa* sp.) is one of the oldest domesticated millets in the semi-arid tropics of Asia and Africa. The genus *Echinochloa* includes some 20 species that are distributed widely in the warmer parts of the world. Two of the main species, *E. crus-galli* and *E. frumentacea* are grown as cereals. In addition to these two domesticated species, the genus includes about 30 annual and biennial wild species distributed worldwide (Clayton and Renvoize, 1986). These millet species are morphologically very dissimilar. Indian barnyard millet (*E. frumentacea*) can easily be distinguished from Japanese barnyard millet (*E. crus-galli*) by its panicle, thinner texture of the glumes and lower lemma (Yabuno, 1971).

The crop is valued for its drought tolerance, good yield and superior nutritional value. It is the fastest growing crop among all millets and can be harvested in a short period of nine weeks. Barnyard millet is an important dual-purpose crop. Its grains contain 6.2 % protein, 9.8 % crude fiber, 65.5 % carbohydrates and are consumed just like rice (Ruiz-santaella *et al.*, 2006). Also it is a nutritive fodder for animals. These aspects make barnyard millet a valuable crop.

But, very little attention has however been paid for the genetic improvement of this crop towards augmenting its yield potential. Heterogeneity of environment and exposure to low temperature at higher elevation causes economic loss in both grain and fodder

yields of barnyard millet cultivars. Moreover, in absence of wide genetic diversity among local cultivars and released varieties, the selection for adaptation to cold temperature is discouraging (Gullord *et al.*, 1975).

Genetic diversity of common morphological traits is difficult to measure in a natural population since the traits are influenced by environmental factors to a large degree. On the other hand, their expression is largely governed by different interacting genes. Complementing the use of morphological markers, molecular techniques for evaluating genetic diversity have been improved in the last decades. Measurement of genetic diversity with molecular markers is relevant to assessment of ecological conditions because it allows estimation of important population parameters e.g., characterization of the geographic structure or connectivity of populations. Molecular markers such as SDS-PAGE and isozymes have been found more useful to study genotypic diversity in many plant species (Tanksley *et al.*, 1989; Paterson *et al.*, 1991).

In the present study we evaluated genetic diversity among 33 different accessions of two cultivated species of barnyard millet (*E. crus-galli* and *E. frumentacea*) on the basis of morphological and biochemical markers. Results would be helpful to identify their role in crop improvement by identifying the diverse accessions.

2. Materials and methods

2.1 Plant material

Two sets of different accessions of both the species (*E. crus-galli* and *E. frumentacea*) were examined. 15 accessions of *E. crus-galli* were procured from ICRISAT, Hyderabad (Andhra Pradesh, India) and 18 accessions of *E. frumentacea* were collected from VPKAS, Almora (Uttarakhand, India).

2.2 Morphological characters

All the 33 genotypes were sown in the fields in a Randomized Block Design (RBD) at G.B.P.U.A. & T., Hill Campus, Ranichauri, Tehri Garhwal (Uttarakhand). Crop management was done according to the recommended agronomic practices. Sowing was done in the plots (3m × 1m), plant to plant distance was maintained 10 cm., while row to row distance was kept 22.5 cm. Eight morphological characters, germination percentage, days to 50% flowering, plant height, no. of fertile tillers, length of spikelet, days to maturity, 1000 grain wt and yield per plant were taken to assess genetic variability in accessions.

2.3 Isozyme analysis

Peroxidase and esterase were isolated to assess the genetic variability. For the isolation of both the isozymes, fresh leaves of plants were collected and washed in tap water followed by distilled water. Water droplets were removed with the help of tissue paper. 500 mg leaves were weighed and crushed in minimum amount of extraction buffer (0.1 M tris + 2% -mercaptoethanol) with the help of mortar and pestle under chilled conditions. The extracts were quickly transferred to pre-cooled eppendorf tubes and kept in a refrigerator. Tubes were centrifuged at 12000 rpm for 30 min at 4°C in a refrigerated centrifuge. The supernatant was transferred to fresh tubes. 1 part

extract: 1 part glycerol: 1 part bromophenol blue (0.05 mg/ml) were mixed and then the samples were stored at -20°C for further use.

The experiments were carried out in order to characterize the genetic variability by Native Polyacrylamide Gel Electrophoresis (PAGE). The staining procedure for peroxidase and esterase isozyme was done according to method by Wendel and Weeden (1989) with minor modifications.

2.4 DATA analysis

Field data for morphological characters was evaluated by the analysis of variance (ANOVA) using RBD to calculate the significance by magnitude of F value (P= 0.01) and D² statistics as suggested by Rao (1952) using computer software. The calculation of D² values involved the steps followed by Murthy and Arunachalam (1996).

For isozyme analysis Pair-wise similarity and cluster analysis were done on the basis of presence and absence of bands. Squared Euclidean distances among the accessions were calculated by computer software (Statistica) to perform the similarity matrix analysis using unweighted pair-group method.

3. Results

3.1 Estimation of genetic diversity based on morphological characters

On the basis of D² analysis, all the accessions were grouped into three clusters (Table-1). Cluster-I comprised of accessions of *E. frumentacea*, cluster-II consists of *E. crus-galli*, while cluster-III had 2 accessions of *E. crus-galli* and rest 10 were of *E. frumentacea* (Table-1).

Table 1: D² statistics* based clustering pattern and average inter and intra cluster d values of the different accessions of two different species of *Echinochloa*.

Clusters	Accessions grouped in different clusters	d values of Clusters		
		I	II	III
I	VRS-MB-1554, VRS-MB-1839, VRS-MB-886, VRS-MB-846, VRS-MB-1361, VRS-MB-1535, VRS-MB-1377, VRS-MB-871 (n=8)	1.76		
II	IEC-530, IEC-531, IEC-540, IEC-545, IEC-546, IEC-547, IEC-548, IEC-549, IEC-555, IEC-556, IEC-542, IEC-533, PRB-9404 (n=13)	3.40	2.25	
III	IEC-535, IEC-538, VRS-MB-1202, VRS-MB-893, VRS-MB-889, VRS-MB-1506, VRS-MB-882, VRS-MB-1546, VRS-MB-1508, VRS-MB-1543, VRS-MB-858, VRS-MB-1372 (n=12)	2.50	3.41	1.91

* The D² analysis was done according to Murthy and Arunachalam, 1996.

Eight different morphological characters (Germination percentage, Days to 50 percent flowering, Plant height, Number of fertile tillers, Panicle length, 1000 seed weight, Days to maturity and Yield per plant) were considered to study genetic diversity.

IEC and PRB: Accession number for the accessions of *E. crus-galli*, VRS-MB: Accession number for the accessions of *E. frumentacea*.

On the basis of D^2 analysis, all the accessions were grouped into three clusters (Table 1). Cluster-I comprised of accessions of *E. frumentacea*, cluster-II consists of *E. crus-galli*, while cluster-III had 2 accessions of *E. crus-galli* and rest 10 were of *E. frumentacea* (Table-1).

The average intra- and inter-cluster genetic distance (d values) shown in Table 1. Inter-cluster centeroids distance ranged from 0.00 to 3.14 and Intra-cluster distance ranged from 1.76 to 1.9. Maximum intra-cluster distance was observed in cluster-II (D=2.25) followed by cluster-III (D=1.91) and cluster-I (D=1.77). Maximum inter cluster centeroids distance was observed in between cluster-III and cluster-II (D=3.41) followed by cluster-I and cluster-II (D=3.40).

The cluster means of various agronomic traits are presented in Table 2. Cluster-III, which consists of 13 accessions, showed maximum average value of germination percentage (92.62 %), followed by cluster-II (91.49 %) and cluster-I (89.83 %). Maximum average value of days to 50 % flowering was shown by cluster-II i.e. 83.65 followed by cluster-III and cluster-I. Maximum average plant height was observed in cluster-III (186.56) followed by cluster-I (173.25) and cluster-II (135.40). Cluster-II showed the maximum mean value of number of fertile tillers (3.43), followed by cluster-III (3.26), while cluster-I showed the least mean value for number of fertile tillers. Maximum average seed weight was shown by cluster-II (4.26), followed by cluster-I (3.51) and cluster-III (2.88).

Table 2: Inter and intra species diversity among *E. frumentacea* and *E. crus-galli* accession on the basis of isozyme (Esterase and Peroxydase) analysis

Accessions	P	A	A _p	H _T
All	54.50	3.28	3	10
<i>E. frumentacea</i>	22.75	3	0.57	6.2
<i>E. crus-galli</i>	45.45	2	0.57	5.4

P- Percent Polymorphic Loci, **A-** Mean number of alleles per locus, **A_p-** Mean number of alleles per polymorphic loci, **H_T-** Total genetic similarity based on linkage distance. Each of the gel was run three times to confirm best reproducible results.

Cluster-II with 13 accessions exhibited highest mean value of days to maturity (128.62) followed by cluster-I (123.71) and cluster-III (123.71). Least mean value of days to maturity was shown by cluster-III. Mean value of highest Yield/ plant was shown by cluster-II (2.06) followed by cluster-III (1.25) and cluster-I (1.16).

3.2 Genetic diversity based on Isozyme markers

A total of 23 alleles were observed at the seven Isozyme loci in all 33 accessions of barnyard millet. Mean number of alleles per locus was 3.28 (Table 2). All allelic distribution is shown in Figure 1,2. Both the species of barnyard millet i.e. *E. frumentacea* and *E. crus-galli* were clearly separated on the basis of both the Isozyme systems.

3.2.1 Peroxidase

Three loci were observed and named as Per-1, Per-2 and Per-3. Per-1 was monomorphic among the studied loci (Figure 1). The other two loci were polymorphic. Two alleles (Per-2^a and Per-2^b) were observed at Per-2 locus. Allele Per-2^a was polymorphic and was present only in the accessions of *E. crus-galli*. Four alleles were detected at Per-3 locus (Per-3^a, Per-3^b, Per-3^c and Per-3^d). Allele Per-3^c was common among all the accessions. Allele Per-3^a was present in all the 15 accessions of the species *E. crus-galli*, while Per-3^c and Per-3^d were present among all 18 accessions of *E. frumentacea*.

3.2.2 Esterases

Esterases showed abundant genetic variability among and within the barnyard species. Four loci (Est-1, Est-2, Est-3 and Est-4) were detected (Figure 2). Locus Est-1 was highly diverse. Four alleles were observed at Est-1 locus (Est-1^a, Est-1^b, Est-1^c and Est-1^d). Est-1^a allele was present in the accessions VRS-MB-886, IEC-535, IEC-538, IEC-548, VRS-MB-893, VRS-MB-846, VRS-MB-889, VRS-MB-1508, VRS-MB-1361, VRS-MB-1372, and VRS-MB-871.

Locus Est-2 showed three alleles i.e. Est-2^a, Est-2^b and Est-2^c. Two alleles (Est-2^a and Est-2^c) were present in all the 18 accessions of *E. frumentacea* and were absent in the accessions of *E. crus-galli*. Five alleles were detected at Est-3 locus ((Est-3^a, Est-3^b, Est-3^c, Est-3^d and Est-3^e). Est-3^a and Est-3^c were polymorphic and present in the accessions of *E. frumentacea*. Total three alleles were detected at locus Est-4 (Est-4^a, Est-4^b, Est-4^c and Est-4^d). Only allele Est-4^c was polymorphic and was present in the accessions of *E. crus-galli*.

Dendrogram was prepared on the basis of banding pattern obtained by both the isozyme patterns. Accessions of both the species (*E. frumentacea* and *E. crus-galli*) were grouped into two groups at a linkage distance of 10 (Fig- 3). Again within the species of *E. frumentacea*, all the accessions were grouped at a linkage distance of 6.2 and were separated into two groups. Other branch of the tree, which separates accessions of *E. crus-galli*, again divided into two subgroups at a 10linkage distance of 5.4.

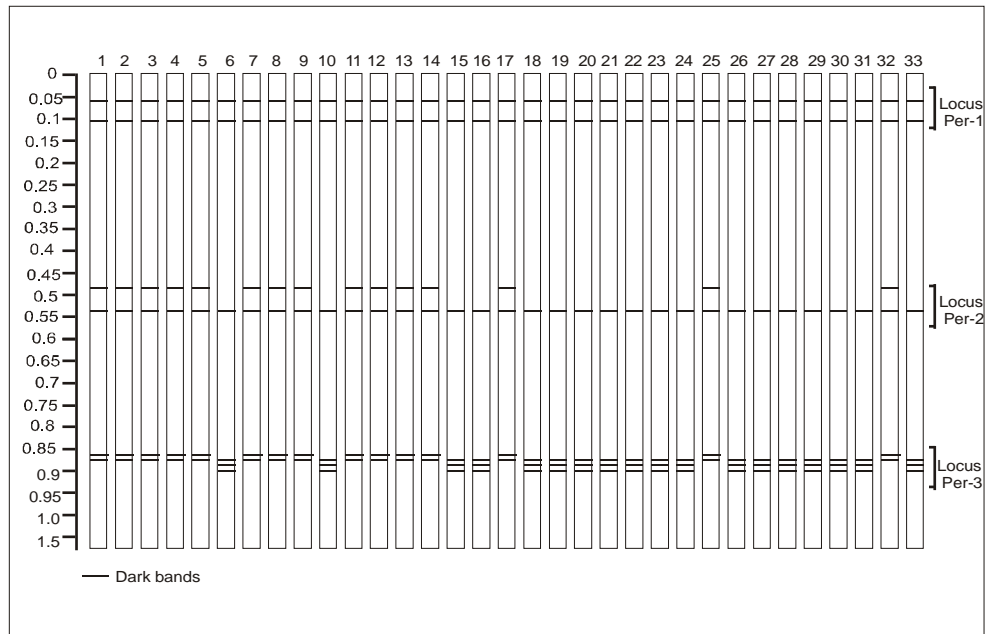


Figure 1: Zymogram of protein profile of thirty-three barnyard millet accessions obtained through peroxidase. (1. IEC-549, 2. IEC-555, 3. IEC-556, 4. IEC-546, 5. IEC-531, 6. VRS-MB-886, 7. IEC-535, 8. IEC-538, 9. IEC-547, 10. VRS-MB-1546, 11. IEC-335, 12. IEC-545, 13. IEC-542, 14. IEC-584, 15. VRS-MB-893, 16. VRS-MB-1377, 17. IEC-530, 18. VRS-MB-1554, 19. VRS-MB-1262, 20. VRS-MB-882, 21. VRS-MB-1506, 22. VRS-MB-1839, 23. VRS-MB-1535, 24. VRS-MB-1542, 25. IEC-543, 26. VRS-MB-846, 27. VRS-MB-889, 28. VRS-MB-1508, 29. VRS-MB-1361, 30. VRS-MB-1372, 31. VRS-MB-871, 32. IEC-533, 33. VRS-MB-1510).

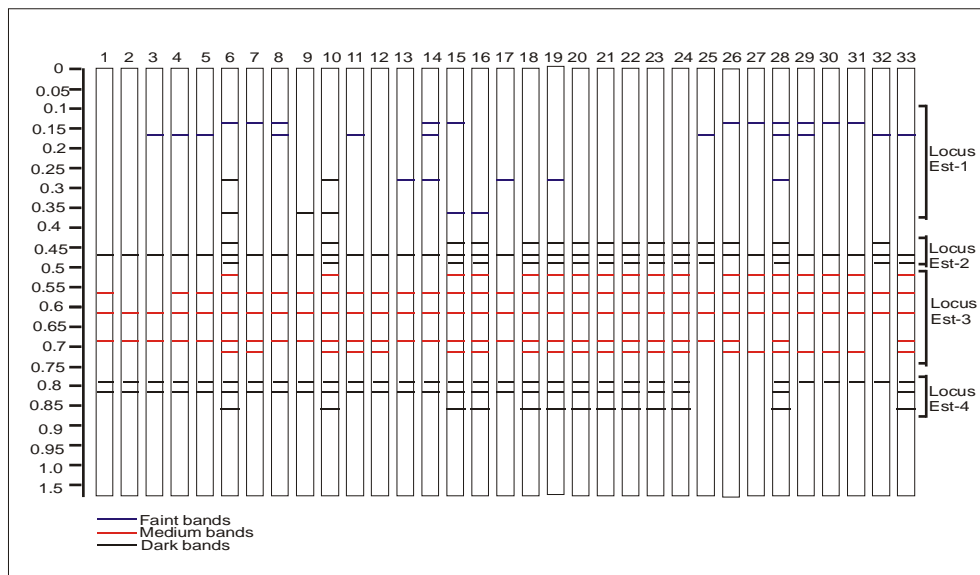


Figure 2: Zymogram of protein profile of thirty-three barnyard millet accessions obtained through esterases. (1. IEC-549, 2. IEC-555, 3. IEC-556, 4. IEC-546, 5. IEC-531, 6. VRS-MB-886, 7. IEC-535, 8. IEC-538, 9. IEC-547, 10. VRS-MB-1546, 11. IEC-335, 12. IEC-545, 13. IEC-542, 14. IEC-584, 15. VRS-MB-893, 16. VRS-MB-1377, 17. IEC-530, 18. VRS-MB-1554, 19. VRS-MB-1262, 20. VRS-MB-882, 21. VRS-MB-1506, 22. VRS-MB-1839, 23. VRS-MB-1535, 24. VRS-MB-1542, 25. IEC-543, 26. VRS-MB-846, 27. VRS-MB-889, 28. VRS-MB-1508, 29. VRS-MB-1361, 30. VRS-MB-1372, 31. VRS-MB-871, 32. IEC-533, 33. VRS-MB-1510).

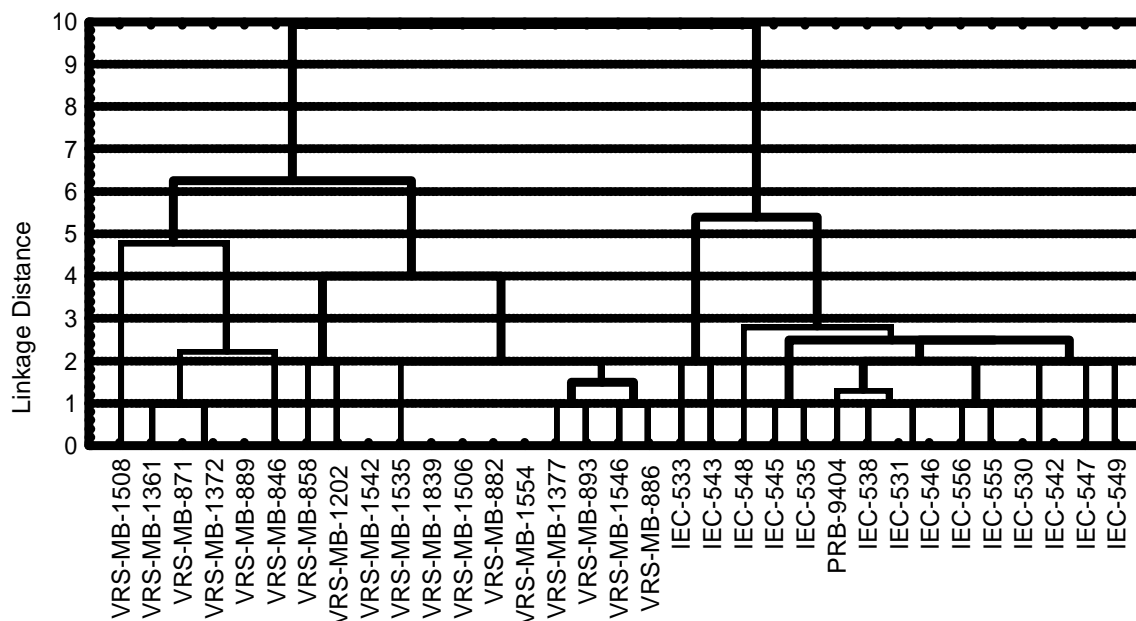


Figure 3: Cluster analysis of 33 accessions of Barnyard millet (belonging to two cultivable species, *Echinochloa crus-galli* and *Echinochloa frumentacea*) on the basis of banding pattern of two isozymes (Peroxidase and Esterase). The unweighted pair-group method, arithmetic mean (UPGMA) algorithm was applied to calculate Squared Euclidean Distance using computer software (Statistica).

4. Discussion

On the basis of D^2 analysis, the 33 accessions were grouped into three clusters (Table-1). Comparison of these morphological clusters revealed that maximum intra-cluster distance ($D=2.25$) was observed in cluster-II whereas, maximum inter cluster centroids distance ($D=3.41$) was observed in between cluster-III and cluster-II (Table 1). It has previously been suggested that genetic drift and selection in different environments can produce greater diversity (Raje and Rao 2001). We observed that accessions of *E. crus-galli* showed genetic superiority to *E. frumentacea* in different agronomic characters. The findings get support by Bandyopadhyay 1998, 1999. He reported that the accessions of *E. crus-galli* were better adapted for Uttarakhand hills and were promising for high grain yield. Average inter-cluster and intra-cluster distance (D) values presented in Table 1 indicated that the maximum variability was present in cluster-II and cluster-III for agronomic characters. So the clusters showing greater genetic diversity can be used in further crop improvement programme. Several workers suggested selection of parents for hybridization from two clusters having wide inter-cluster and intra-cluster distance to get maximum heterosis (Pradhan and Rao 1990; Mehta et al., 2005).

In isozyme analysis, peroxidase was able to differentiate both the species but, it was not able to differentiate accessions of the same species. Among the three loci genetic diversity per locus for peroxidase followed the trend, Per-3> Per-2> Per-1. on the other hand, esterases showed large number of alleles and polymorphism to differentiate the two species as well as accessions of the same species. Maximum diversity was observed in locus Est-1, which differentiated accessions, while the other loci differentiate two species (*E. frumentacea* and *E. crus-galli*).

Variation at isozyme loci revolutionized the research and evolution (Schwartz 1969; Wendel and Weeden 1989; Sachs et al., 1996.). Only the polymorphic bands are actually of use in genetical, physiological or taxonomical studies (Bassiri 1976). Similarity coefficient based on the Isozyme data showed that the linkage distance ranged from 0 to 10. Grouping of accessions near to 0 indicates the close similarity among them, whereas the increase in linkage distance to 9.9/10 tends to differentiate two species into two groups. Group 1 consisted of all the accessions of *E. frumentacea*, whereas accessions of *E. crus-galli* were grouped into group 2. The findings of our study get support by Werth et al. (1994). They studied the isozyme variation in finger millet species and reported the distinct allelic composition between two species.

The maximum variability was present in cluster-II and cluster-III for agronomic characters. The accessions from these clusters can be used in further hybridization programme. In isozyme analysis, peroxidase was able to differentiate both the species but, it was not able to differentiate accessions of the same species. Esterases showed large number of alleles and polymorphism to differentiate the two species as well as accessions of the same species.

Selection of better species can be made for species improvement on the basis of percent similarity with other species. Two more similar but possessing distinct characters can be chosen for the purpose.

Acknowledgment: We are thankful to Dr. R. Prasad, Dr. Arun Kumar for providing seed material, Dr. Usha for assistance.

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References

1. Bandyopadhyay BB. A study on variability in sink capacity and grain yield of barnyard millet (*Echinochloa frumentacea*). *Annals of Agriculture Research* 1998; 19:34-8.
2. Bandyopadhyay BB. Genotypic difference in relation to climate adaption of two cultivated barnyard millet species at Garhwal hills. *Indian Journal of Genetics and Plant Breeding* 1999; 59: 105-8.
3. Bassiri A. Barley cultivar identification by use of Isozyme electrophoretic patterns. *Canadian Journal of Plant Science* 1976; 56: 1-6.
4. Clayton W D, Renvoize S A. Genera Geranium. *Kew bull. Add. Ser.* 1986; 13: 280-1.
5. Gullord M, Olien CR, Everson EH. Evaluation of freezing hardness in winter wheat. *Crop Science* 1975;15:153-7.
6. Murthy BR, Arunachalam V. The nature of genetic divergence in relation to breeding system in some crop plants. *Indian Journal of Genetics* 1996;26: 188-8.
7. Paterson AH, Tanksley SD, Sorrels ME. Marker in plant improvement. *Advances of Agronomy* 1991; 46: 39-90.
8. Rao B, Murty VAR, Subramanya RJ. The amylase and amylopectin content of rice and their influence on cooking quality of cereals. *Proc. Indian Academy of Science* 1952; 36: 70-4.
9. Ruiz-Santaella, Bastida JP, Franco F, De Prado RAR. Morphological and Molecular Characterization of Different *Echinochloa* spp. and *Oryza sativa* Populations. *J. Agric. Food Chem* 2006;54:1166-72.
10. Schwartz D. An example of gene fixation resulting from selective advantage in suboptimal conditions. *American Naturalist* 1969; 103:479-81.
11. Tanksley SD, Young ND, Paterson AH, Bonierbale MW. RFLP mapping in plant breeding: new tool for an old science. *Biotechnology* 1989;7:257-64.
12. Wendel JF, Weeden NF. Visualization and interpretation of plant isozymes. In : Soltis, D. E., Soltis, P. S., (Eds.), *Isozymes in plant biology*. Discorides, Portland, OR, 1989; pp. 5-45
13. Werth CR, Hilu KW, Langher CA. Isozyme of *Eleusine* (Graminae) and the origin of finger millet. *American Journal of Botany* 1994;18:1186-97.
14. Yabuno T. A note on barnyard millet. *SABURAO News letter* 1971; 3:43-5.

5/5/2010