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Molecular characterization of elite finger millet (*Eleusine coracana* Gaertn.) accessions

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SUMMARY

RAPD primers are used to analyze the genetic diversity of an individual by using random primers. In this study eight finger millet genotypes resistance to neck and finger blast diseases were used and 14 random amplified polymorphic DNA (RAPD) markers were run, which has resulted in 71 different levels of amplified products with an average of 5.07 bands per primer. Out of 71 profile levels, 63 levels were polymorphic across eight finger millet accessions and eight levels were monomorphic. The markers detected significant polymorphism among genotypes and genotype specific markers were identified. Cluster analysis grouped the eight finger millet accessions into two major clusters. The Indian types were grouped with African type accessions in the study.

Key words : RAPD, Random amplified polymorphic, DNA, Finger millet

Finger millet (*Eleusine coracana* Gaertn.) is an important food crop in Africa and South Asia. It is a hardy crop that can be grown in diverse environments from almost at sea level in South India to high lands of Himalaya. It is believed that Uganda is the centre of origin and it was introduced to India probably over 3000 years ago (FAO, 1995). Finger millet is highly nutritious as its grains contain 65–75% carbohydrates, 5–8% protein, 15–20% dietary fiber and 2.5–3.5% minerals (Chetan and Malleshi, 2007).

The efficiency of selection based on phenotype assay may be reduced by environmental effects on the measured trait and by complex inheritance of multigenic traits. Many of the complications of phenotype based selection can be mitigated by direct selection for genotype using DNA markers that co segregate with genes of interest (Antoni *et al.,* 1991).There are many number of DNA markers for characterization among different crop species. The simplicity and applicability of the RAPD technique have captivated many scientists interests. Perhaps the main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the requirement for cloning, sequencing or any other form of the molecular characterization of the genome of the species in question (Fevzi Bardakci, 2001)

AFLP requires a genomic DNA of high purity and having an advantage of small sequence variation can be detected (Blears *et al.,* 1998). In RFLP radioactive probe is used and have an advantage that they can be used to detect multiple alleles at a single locus (Antoni *et al.,* 1991). SNP is the polymorphism occurring between DNA samples with respect to single base. SNP primers are good for studying complex genetic traits and for understanding the genomic evolution (Tabassum and Lakhanpaul, 2006). RAPD markers offer many advantages such as higher frequency of polymorphism, rapidity, technical simplicity, use of fluorescence, requirement of only a few nano grams of DNA, no requirement of prior information of the DNA sequence and feasibility of automation (Subudhi and Huang, 1999). RAPD marker having an added advantage over microsatellite due to no requirement of prior sequence information.Eight accessions from the collections were selected based on resistant reaction to neck and finger blast diseases with higher grain yield and other quantitative traits of economic importance. Molecular characterization of these elite collections is necessary to initiate marker assisted selection programme. Different kinds of markers have been utilised to assess diversity. Random Amplified Polymorphic DNA (RAPD) is one such marker system used in finger millet to assess

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genetic diversity (Salimath *et al.*, 1955 and Fakrudin *et al.*, 2004). The objective of the study was to assess the molecular and morphological diversity among eight finger millet accessions.

The present study was conducted to know the extent of genetic diversity among eight finger millet accessions which were used as parents in hybridization programme at Zonal Agricultural Research Station, V.C. Farm, Mandya, Karnataka, India.

MATERIALS AND METHODS

More than 1000 accessions of finger millet are being maintained at Zonal Agricultural Research Station, V.C. Farm, Mandya, Karnataka, India, collected from different geographical locations in India and Africa. All the elite eight accessions were grown in pots in green house facilities at Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru. The extraction of DNA was carried out using CTAB method. The concentration and quality of DNA was estimated using 0.8% agarose gel electrophoresis. Physical and chemical factors for better amplification using random primers were worked out by trial and error before beginning the objective. The RAPD reaction mixture consisted of 50 ng of template DNA, 20 ng of random decamer perimer (operon, USA), 1 mM of dNTPs, 0.6 µl of Taq polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). 10 X PCR buffer (10 mM Tris pH 8.0, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) in a volume of 25 µl. One drop of mineral oil (Sigma) was dropped on reaction mixture. Amplification was carried out in a MJ Research PTC 200 Thermal cycler. The amplified PCR product was electrophoresed. The bands were scored as '1' for presence of band '0' for absence of band. The data was later used for further analysis. Whole set of data generated by the detection of polymorphic fragments were used for calculating an index of genetic similarity of distance. The computer package programmed for Euclidean distance was used, which simply gives the geometric distance in the multidimensional space. It is computed as

$$(X, Y) = \{Ói (Xi-Yi)^2\}^{1/2}$$

An agglomerative method of clustering accessions was employed utilizing the unweighted pair group method with arithmetic mean (UPGMA).

RESULTS AND DISCUSSION

The molecular survey of the accessions by RAPD using 14 random primers resulted in 71 different levels of amplified products. Out of 71 profile levels, 63 levels were polymorphic across eight finger millet accessions and eight levels were monomorphic (Table 1). The primers OPB-4 (5'-GGACTGGAGT-3') and OPA-5 (5'-AGGGGTCTTG-3') generated maximum eight levels of bands while OPA-3 (5' -AGTCAGCCAC-3') generated six levels. A minimum number of two levels were produced by primer OPO-11(5' -GACAGGAGGT-3'). On an average, 5.07 levels of polymorphic bands per primer were recorded.

Table 1 : Summary statistics of RAPD analysis of eight finger millet

Parameter	Estimate
Total marker levels	71
Total number of polymorphic levels	63
Average number of markers per primer	5.07
Maximum number of bands produced by a primer	8
Minimum number of bands produced by a primer	2
Average number of polymorphic bands per primer	4.5

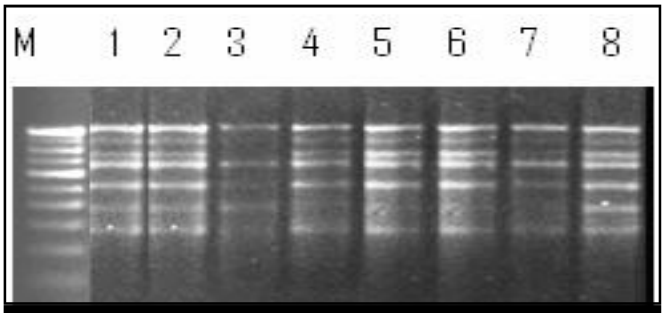

Fig. 1 : RAPD banding profile of eight finger millet genotypes using primer OPB 4
M- Molecular marker, 1- IE 2656, 2- INDAF 5, 3- IE 2712, 4- IE 2936, 5- GE 1409, 6- L 5, 7- GPU 26 and 8- L 264

The summary statistics of the results are presented in Table (1) and the molecular phylogenic dendrogram in Fig. 2.

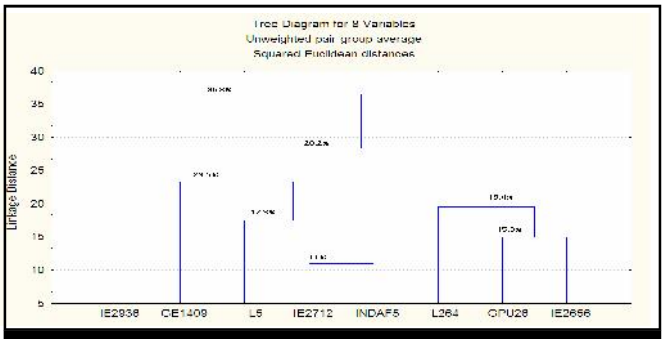

Fig. 2 : Dendrogram constructed from the RAPD data of finger millet accession

Two major clusters (X_1 and X_2) were resolved corresponding to African and Indian ecotypes with two accessions intermixed in each cluster. X_1 cluster included GE 1409, L 5, IE 2712, and Indaf 5. GE 1409 was having

23.5per cent dissimilarity from the remaining set of genotype; L 5 were 17.3% dissimilarity from IE 2712, Indaf 5 and IE 2712 was 11% dissimilarity from IE 2712. X_2 cluster involved L 264, GPU 26 and IE 2656. L 264 is having 19.4 per cent of dissimilarity index with the remaining and GPU 26 was 15.3% dissimilarity with IE 2656. When compared both to the cluster, one of the African type IE 2656 was clustered into Indian group, while 2 of Indian types namely, Indaf 5 and L 5 was grouped with African type. In the range of 36.3per cent dissimilarity index to the maximum, no other genotype shares homology with IE-2936. Indian and African genotypes were intermixed between clusters. Similarity index values arrived from the polymorphic data gave the extent of relatedness between genotypes. Higher the dissimilarity between the genotypes, better the scope to include them in hybridization programmes (Kalyana babu *et al*., 2007). When the field data of Indaf-5 and GE-1409 were compared, both the genotypes varied considerably with respect to blast disease and weight of main ear. GE-1409 was resistant to blast disease with lower ear weight, while Indaf-5 was having moderate susceptibility to blast disease with high weight of main ear. Finally RAPD is a use full molecular tool to find out the diversity at genetic level. From this study, the two parents GE-1409 and Indaf-5 can be used as parents for further hybridization programme for better yield and blast resistant finger millet improvement.

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