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ASSESSMENT OF GENETIC DIVERSITY AMONG FINGER MILLET GENOTYPES USING RAPD MARKERS

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ABSTRACT

Genetic diversity among fifteen finger millet genotypes was estimated using RAPD markers. Out of the initial twenty-five random primers, nine RAPD primers were found to be highly reproducible and produced a total of 60 loci of which 51 loci were polymorphic. Primer OPC18 amplified highest number of polymorphic bands. Percentage of polymorphism varied from 50% to 100% with an average of 80.8%. Polymorphism information content (PIC) value of the primers ranged from 0.17 to 0.38 and the resolving power (Rp) varied from 2.66 to 12.06. Primers OPN16 and OPD8 exhibited high Rp along with high PIC value. The coefficient of similarity in banding pattern among genotypes ranged from0.42 to 0.89. The genotypes OEB 65 & HR 374 and BM 107 & SRS 2 showed high genetic similarity of 0.89 and 0.87, respectively indicating low genetic diversity and also showed similarity in growth traits. DM 7 and AKP 2 showed the lowest similarity of 0.42 indicating them to be quite diverse. Cluster analysis by UPGMA method grouped the fifteen finger millet genotypes into one major cluster of eight genotypes and five minor clusters indicating presence of genetic diversity among the genotypes at molecular level.

Key words: Finger millet, Genetic diversity, RAPD markers

INTRODUCTION

Finger millet or ragi is the most important of the small millets grown for food. It is an important food crop in South Asia and Africa. In India, several kinds of traditional food are prepared from ragi grains. Finger millet is nutritionally comparable or even superior to major cereals in respect of protective nutrient content. It is a rich source of calcium and has good amounts of magnesium, phosphorus and iron. Finger millet protein has a favourable amino acid spectrum that includes cystine, tyrosine, tryptophan and methionine (Rachie, 1975). Genetic improvement of the crop depends on the amount of genetic variability present in the population. Though several methods have been developed to measure the genetic diversity, the molecular approach is more efficient as it directly quantifies genetic variability at DNA level. Molecular markers have also provided a powerful tool for breeders to search for new sources of variation and to investigate genetic factors controlling quantitatively inherited characters. PCR-based molecular markers have been widely used in many plant species including finger millet for identification, phylogenetic analysis, population studies and genetic linkage mapping (Salimath *et al.* 1995). The RAPD markers can also be used in the study of genetic variability of species or natural populations (Wilkie *et al.* 1993). In the present study an attempt has been made to assess genetic variability present among fifteen finger millet genotypes by using RAPD markers.

MATERIAL AND METHODS

Fifteen finger millet genotypes collected from different places of India were used for the present

study. Table 1 gave detailed information about the material used, their place of collection, plant height, maturity duration and yield. The experiment was conducted in Department of Plant Breeding and Genetics, OUAT, Bhubaneswar during July- August, 2005. DNA extraction was done following Cetyl-trimethly Ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1990). For each genotype, 20 mg of young leaves was collected from 9-day old seedlings, cut into small pieces, grinded in liquid nitrogen and homogenized. 10 ml of preheated extraction buffer (2 % CTAB, 0.2 % β-mercaptoethanol, 100 mM Tris-HCl of pH 8.0, 2 mM EDTA, 1.4 M NaCl) was added per 20 mg of powdered material and incubated for 2 hours at 60°C. After incubation, an equal amount (10 ml) of isoamyl alcohol: chloroform (24:1) was added and centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and precipitated by using equal volume of chilled isopropanol. Crude DNA pellet was made by adding chilled ethanol. The DNA pellet was resuspended in 200 µl of Tris-EDTA (TE) buffer (10 mM Tris-HCl of pH 8.0 and 0.1 mM EDTA) for overnight. To remove contaminant RNA, the sample was treated with 3 µl RNase (10 mg/ml) and then incubated in water bath for 1 hour at 37°C. DNA purification was done two times by using equilibrated phenol. The pure DNA was reprecipitated with 0.1 volume of 3 M sodium acetate and 2 volumes of cold absolute ethanol. The precipitated DNA was spooled, rinsed with 70% ethanol and dissolved in 1 ml of TE buffer for further analysis. After electrophoresis on 0.8 % (w/v) agarose gel DNA quantification was done by visualizing under UV light. The DNA was again diluted in TE buffer to 5 mg/ml concentration for use in polymerase chain reactions.

Twenty-five decamer primers of A, C, D and N kits of Operon Technologies (Alameda, California, USA) were used for initial screening. Primers producing distinct, clear polymorphic amplified products were selected for PCR study. Polymerase chain reactions were carried out in a final volume of 25 ml containing 20 ng template DNA, 100 µM each deoxy-nucleotide triphosphate, 20 ng of primers, 1.5 mM $MgCl_2$, 1x Taq buffer (10 mM Tris-HCl of pH 9.0, 50 mM KCl, 0.01 % gelatin) and 0.5 U Taq DNA polymerase. Amplification was achieved in a PTC-100 thermal cycler (M.J. Research, USA) programmed for a preliminary 2-minute denaturation step at 94°C, followed by 40 cycles of denaturation at 94°C for 20 seconds, annealing at 38°C for 1 minute and extension at 72°C for 2 minutes and finally at 72°C for 10 minutes. Amplification products were separated alongside a molecular weight marker (100 kb ladder, MBI Fermentas, USA) by electrophoresis on 1.2 % agarose gel run in 0.5x TAE (Tris Acetate EDTA) buffer, stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through Gel Doc system (Gel Doc, 2000, BioRad, USA) and the amplification product sizes were evaluated using the software Quantity (Bio Rad).

Data analysis

The photographic negatives were examined for each amplification bands and data on presence (1) or absence (0) of the bands in each genotype was recorded. Each amplification fragment was named by the source of the primer (Operon, Advanced Biotechnologies), the kit letter or number, the primer number and its approximate size in base pairs. Bands with similar mobility to those detected in the negative control, if any, were not scored. Similarity indices among genotypes were estimated using the Dice coefficient of similarity (Nei and Li, 1979). Cluster analysis of genotypes was carried out on similarity estimates using the unweighted pair-group method arithmetic average (UPGMA) using NTSYS-PC, version 1.80 (Rohlf, 1995).

The discriminatory power of RAPD marker was evaluated by 2 parameters. The polymorphic information content (PIC) for each RAPD marker was calculated as PICi = 2fi(1-fi) as proposed by Roldan-Ruiz *et al.*, 2000, where PICi is the polymorphic information content of the marker i, fi is the frequency of the marker bands present, (1-fi)

is the frequency of absent marker bands. PIC was averaged over the bands for each primer. The resolving power of the 9 primers was calculated as $Rp = I_b$ where, I_b (band informativeness) takes the value of $1- [2 \times (0.5 – p)]$, p being the proportion of genotypes containing the band (Prevost and Wilkinson, 1999).

RESULTS AND DISCUSSION

Molecular characterization of the fifteen finger millet genotypes was done through RAPD markers. Twenty-five decamer primers were used for initial screening out of which nine primers (OPA4, OPA13, OPA16, OPC12, OPC18, OPD8, OPN7, OPN15 and OPN16) which were highly reproducible and developed more bands were selected for to analyzing the genetic relationship among the fifteen genotypes of finger millet. The reproducibility of the amplification product was tested with three independent extractions. Most of the amplification reactions were duplicated. The bands that were consistently reproduced across amplifications were considered for the analysis. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. Therefore, 9 informative primers were selected and used to evaluate the genetic variability present within fifteen finger millet genotypes. The pattern of RAPD banding produced by the 3 primers OPN15, OPA13 and OPD8 are shown in Fig.1.

Table 2 gives detailed information about the primers used, total number of bands (TNB), number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), number of unique bands (NUB), polymorphism information content (PIC), resolving power (Rp) and average informativeness (Av. Ib). Number of bands produced by primers ranged from 2 to 9 with an average of 6.7 bands per primer. The nine primers produced a total of 60 amplification products (bands) of which 9 were monomorphic and 51 were polymorphic (85%). Yu and Nguyen (1994) detected 80% polymorphic RAPD bands in thirteen rice cultivars. The primers OPA4, OPC18 and OPN15 produced greater number (8-9) of polymorphic bands, while OPA16 and OPN16 produced only 1 and 3 polymorphic bands, respectively. All the nine primers were effective in bringing out differences among the 15 finger millet genotypes. Different primers showed variation in their ability to detect polymorphism. The percentage of

Table 1: Place of collection, maturity duration, plant height, and yield of 15 finger millet genotypes

Name of genotype	Place of collection	Maturity duration(days)	Plant height(cm)	Yield(q/ha)
V1. Bhairabi	Orissa	102	100	24.5
V2. Dibyasinha	Orissa	91	80	16.1
V3. Neelachal	Orissa	101	117	24.1
V4. OEB 65.	Orissa	99	104	21.7
V5. VL 149	Almora	96	109	21.5
V6. RAU 8.	Bihar	104	80	24.1
V7. VR 708	AP	95	77	17.5
V8. BM-107-2.	Orissa	99	103	25.1
V9. SRS-2	Orissa	107	98	24.7
V10. KM 231	UP	97	78	18.0
V11. HR 374	Karnatak	102	102	21.8
V12. PES 400	Pantnagar	98	86	18.0
V13. VL 322	Almora	99	82	17.7
V14. DM 7	Dholi	106	91	24.1
V15. AKP 2	AP	89	77	13.6

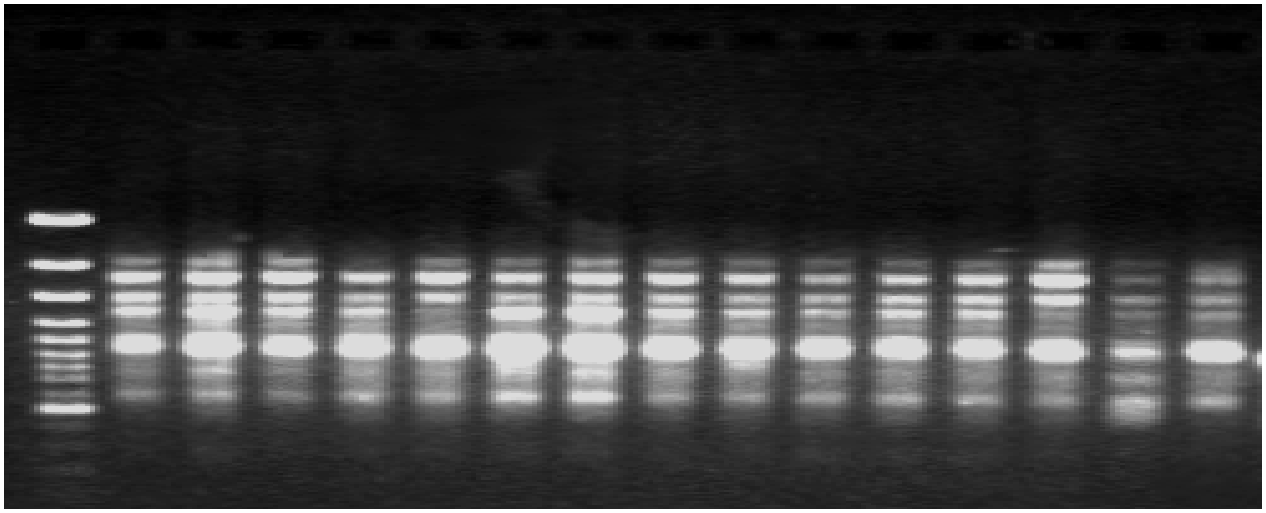
Table 2: Polymorphism exhibited by RAPD primers

Primer	TNB	PB	PPB	NUB	PIC	R_p	Av. I_b
OPA4	9	8	88.8	2	0.31	6.91	0.77
OPA13	6	6	100	0	0.29	8.44	1.41
OPA16	2	1	50	0	0.22	2.66	1.33
OPC12	7	6	85.7	1	0.18	10.38	1.48
OPC18	9	9	100	2	0.21	12.06	1.34
OPD8	7	6	85.7	1	0.26	8.77	1.25
OPN7	6	4	66.7	0	0.25	8.11	1.35
OPN15	8	8	100	0	0.38	11.03	1.38
OPN16	6	3	50	0	0.17	10.38	1.73
Average	6.67	5.67	80.8	0.66	0.25	8.74	1.34

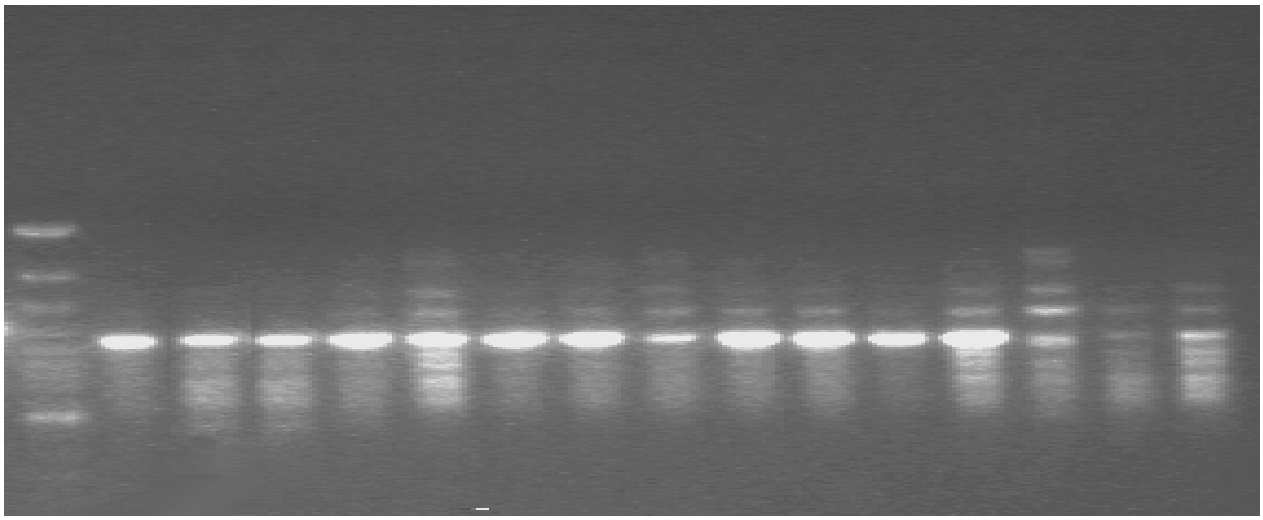
polymorphism ranged from 50 to 100%. Primers OPA13, OPC18 and OPN15 revealed 100% polymorphism where as OPA16 and OPN16 showed 50% polymorphism. Primers OPA4, OPC12, OPC18 and OPD8 amplified 6 unique bands in genotypes VL 322, Neelachal and VL 149. VL 322 showed 4 unique bands where as rest two had 1 unique band each. The 9 primers exhibited variation with regard to their PIC, Av. Ib and Rp values. The PIC value of the primers ranged from 0.17 (OPN16) to 0.38 (OPN15) with an average of 0.25 per primer The Rp values varied from 2.66 (OPA16) to 12.06 (OPC18) with an average of 8.74 per primer. The RAPD primers OPC18 OPN15, OPN16, OPC12 and OPD8 possess high Rp values of 12.06, 11.03, 10.38,0.38 and 8.77, respectively. The primers with higher Rp values were able to distinguish more number of genotypes. Primers having high Rp along with high PIC values are more suitable for analysis of genetic diversity (Devanshi et al., 2007). In the present study the primers OPD8 and OPN15 had high Rp and high PIC values. Hence these two primers could give more information on diversity study. The Av.Ib value of the primers varied from 0.77 to 1.73 with a mean of 1.34. Studies on discriminatory power of RAPD primers have not been carried out in finger millet. But similar studies have been done in other self pollinated crop such as barley. A highest PIC value of 0.52 was obtained in barley (Russell *et al.,* 1997), which was higher than the highest PIC value of of 0.38 in the present study. Lowest Rp value of 3.85 was reported in barley (Fernandez *et al.*, 2002) that was similar to 2.66 in this study.

The similarity matrix was obtained using Nei and Li's Dice coefficient of similarity. The coefficient of similarity among genotypes ranged from0.42 to 0.89. The genotypes OEB 65 & HR 374 and BM 107 & SRS 2 showed high genetic similarity of 0.89 and 0.87, respectively indicating low genetic diversity and also showed similarity in growth traits. DM 7 and AKP 2 showed the lowest similarity of 0.42 indicating them to be quite diverse. The similarity matrix was then used to construct a dendrogram with UPGMA method (Fig.2). The dendrogram showed one major cluster of 8 genotypes and five minor clusters.

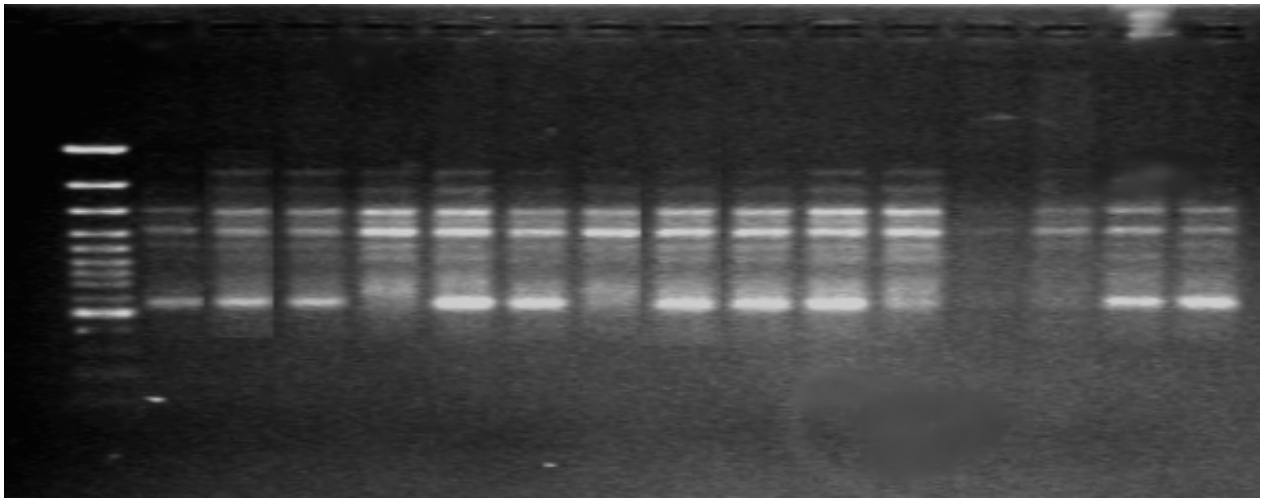
The major cluster consisting of 8 genotypes was again divided into two sub major clusters-having 4 genotypes each. Genotypes VR 708, KM 231, BM 107-2 and SRS-2 were present in first sub major cluster. The genotypes BM 107-2 and SRS- 2 of the first sub major cluster showed 87% similarity with each other due to their same geographic distribution and high yield potential. The genotypes OEB65, HR 374, Bhairabi and Dibyasinha were included in second sub major cluster. Among these



OPN 16



OPA 14



OPN 15

Fig. 1: RAPD banding patterns of 15 finger millet genotypes generated by using primers

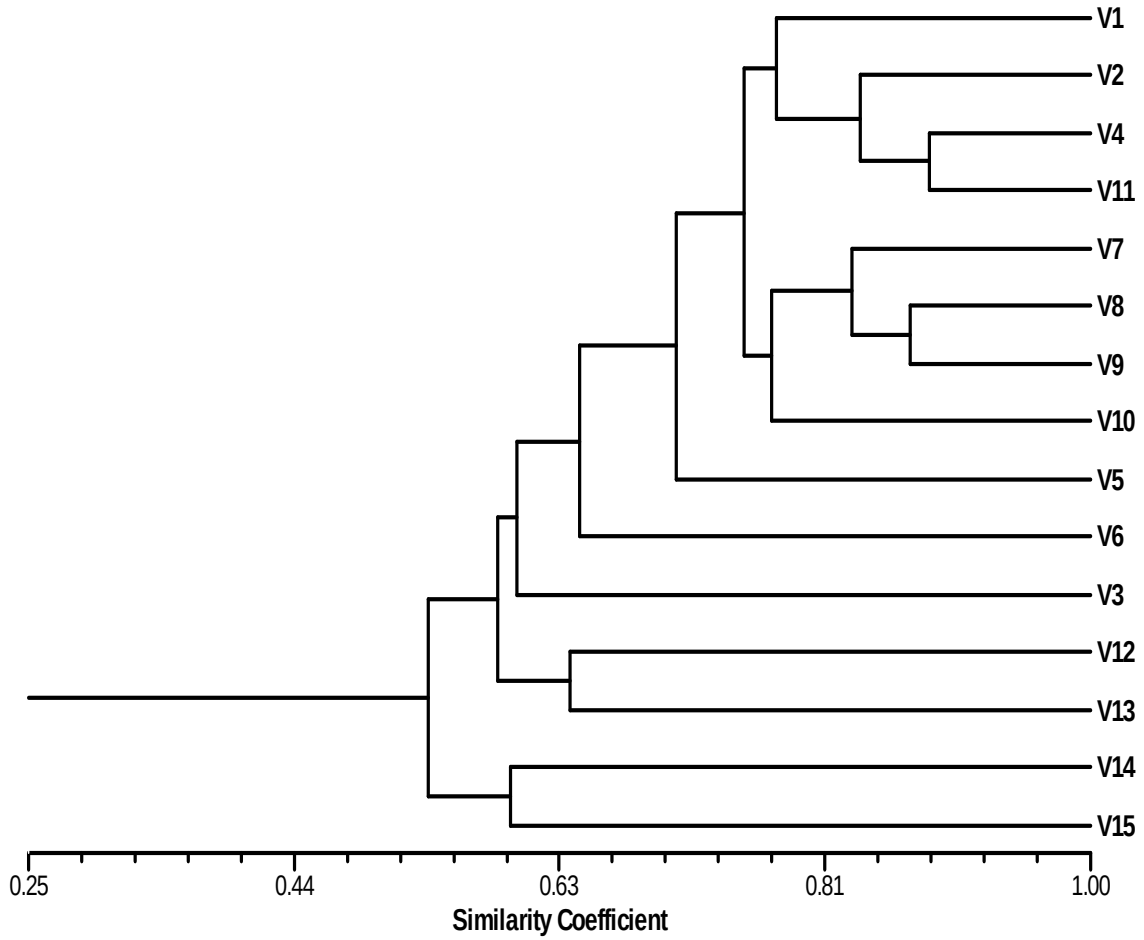


Fig.2: Dendrogram of 15 finger millet genotypes generated by UPGMA based on banding pattern using RAPD markers

four genotypes, OEB 65 & HR 374 exhibited maximum similarity (89%) and was similar with each other in respect of their plant height, maturity duration and yielding ability but different in their geographic distributions.

The first minor cluster contained two genotypes namely DM7 and AKP2 with genetic similarity (59%) at molecular level. The second minor cluster consisted of two genotypes- PES 400 & VL 322 with a genetic similarity of 63%, had similar plant height, maturity duration and low yield potential but different geographic origins. The rest three minor clusters consisted of single genotypes each i.e. Neelachal, RAU 8 and VL 149. These five minor clusters not only showed diversity at molecular level but also contained genotypes with diversity in plant growth traits and geographic origin. The genetic variation through molecular markers has been highlighted in a number of cereal crops (Parani *et al.*, 2001). The pattern of genetic variation was closely correlated to geographic distribution (Hillu, 1995).

The results of the present study suggest the efficiency of RAPD markers in investigating genetic relationship at molecular level, which is important for germplasm conservation and varietal identification. This would also help breeders in choice of parents for hybridization programme.

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