Genetic Variability and Relationships among Thirty Genotypes of Finger Millet (*Eleusine coracana* L. Gaertn.) Using RAPD Markers

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Ragi or finger millet (*Eleusine coracana* L.) is an important crop used for food, forage, and industrial products. It is distributed in tropical and temperate regions of the world. The germplasm identification and characterization is an important link between the conservation and utilization of plant genetic resources. Traditionally, species or varieties identification has relied on morphological characters like growth habit, leaf architecture or floral morphology. Investigation through RAPD (random amplified polymorphic DNA) markers was undertaken for identification and determination of the genetic variation among thirty genotypes of ragi of the family Poaceae. Thirteen selected decamer primers were used for genetic analysis. A total of 124 distinct DNA fragments ranging from 300–3000 bp was amplified by using selected random RAPD marker. The genetic similarity was evaluated on the basis of the presence or absence of bands. Cluster analysis was made by the similarity coefficient. It indicated that the 30 genotypes of ragi form two major clusters, first, a major cluster having only one genotype, *i. e.* Dibyasinha and a second major cluster having twenty-nine genotypes. The second major cluster again subdivides into two minor clusters. A first minor cluster has only three varieties, *i. e.* Neelachal, OEB-56 and Chilika. The genotypes Neelachal and OEB-56 exhibit a 86% similarity with each other and 80% similarity with Chilika. A second minor cluster has 26 genotypes and is divided into two sub-minor clusters. The first sub-minor cluster has only one genotype (VL-322). The second sub-minor cluster again subdivides into two groups. One group has one genotype and the second group again is divided into two sub-groups, one with 13 genotypes and the other with 11 genotypes. The highest similarity coefficient was detected in a genotype collected from southern India and the least from northern India. The genotypes of finger millet collected from diverse agroclimatic regions of India constitute a wide genetic base. This is helpful in breeding programs and a major input into conservation biology of cereal crop.

Key words: Finger Millet, Genetic Variation, RAPD Markers

Introduction

Finger millet (*Eleusine coracana*), an annual allotetraploid cereal, is widely cultivated in the arid and semiarid regions of the world and belongs to the family Poaceae. It is distributed in Eastern Africa and South Asia, particularly in India (Hilu and de Wet, 1976). Identification and utilization of diverse germplasm is the important issue in plant breeding. Information on genetic diversity of the crop is necessary for parental selection that maximizes genetic improvement. Numbers of varieties with origin concerning the evolution of domesticated and wild annual species of *Eleusine* are not properly documented. During the past decade, phenotypical characteristics have been used for classification and identification of species/varieties. Taking into account the utility, the conservation of genetic diversity and building up of nuclear base populations are essential for the improvement of cereal crops. The most important role of conservation is to preserve the genetic variation and evolutionary process in viable populations of ecologically and commercially viable species/varieties/genotypes in order to prevent potential extinction. Molecular markers have provided a powerful new tool for breeders to search for new sources of variation and to investigate genetic factors controlling quantitatively inherited traits. The molecular approach for identification of plant varieties/genotypes seems to be more effective than traditional morphological markers because it al-

lows direct access to the hereditary material and makes it possible to understand the relationships between plants (Williams *et al*., 1990; Paterson *et al*., 1991). 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 (Williams *et al*., 1990; Hilu, 1995; Salimath *et al*., 1995). The RAPD markers can also be used in the study of the genetic variability of species or natural populations (Lashermes *et al*., 1993; Wilkie *et al*., 1993) and in the identification of genotypes (Wilde *et al*., 1992; Koller *et al*., 1993; Wolff and Peters-Van Run, 1993). In this communication, we report the identification and genetic variation among thirty genotypes of ragi (finger millet) from India by using RAPD markers.

Materials and Methods

Plant material

Thirty genotypes of finger millet (*Eleusine coracana* L. Gaertn.) were collected from different germplasm center of India. The origin, yield and collection status of the Indian genotypes are presented in Table I.

Table I. Details of collection of finger millet (*Eleusine coracana*).

Sl. No.	Name of genotype	Parentage	Region of collection	Maturity duration [d]	Average yield [Q/ha]
V1	Bhairabi	Induced mutant of Bhuda mandia local	Orissa	100	27.4
V2	Chilika	GE-68 × GE-156	Orissa	115	26.0
V3	Dibyasinha	Mutant of AKP-7	Orissa	95	20.0
V4	Neelachal	Mutant of IE-642	Orissa	110	27.0
V5	OEB-56	GPU-26 × L-5	Orissa	112	25.0
V6	OEB-65	TNAU-896 × Co-7	Orissa	105	19.1
V7	OEB-71	TNAU-533 × Co-13	Orissa	122	22.2
V8	PR-202	Pure line selection from dry ragi of Araku valley local	Andhra Pradesh	115	30
V9	VL-149	VL-201 × IE-882	Almora	106	25.0
V10	RAU-8	BR-407 × Ranchi local	Bihar	105	28.0
V11	VR-708	Pure line selection from VMEC-36	Andhra Pradesh	97	18.5
V12	BM-107-2	Mutant from B7-7-43	Orissa	110	24.6
V13	SRS-2	Pure line selection from Koraput local	Orissa	108	24.7
V14	KM-231	Pure line selection from Kanpur local	Uttar Pradesh	100	19.5
V15	HR-374	EC-4840 × IE-927	Karnatak	109	15.0
V16	PES-400	Pure line selection from local germplasm	Pantnagar	106	23.3
V17	VL-322	IE-3732 × VL-137	Almora	103	23.5
V18	DM-7	Pure line selection from Dholi local	Dholi	109	23.0
V19	GPU-57	GE-4903 × Sel-14	Bangalore	123	27.0
V20	PES-110	Selection from germplasm	Pantnagar, Punjab	117	26.5
V21	VR-849	GPU-26 × L-5	Andhra Pradesh	110	27
V22	VR-822	GPU-30 × PPR-2614	Andhra Pradesh	119	26
V23	MR-33	Indaf-5 × IE-4541-2	Madhya Pradesh	112	25.8
V24	VR-768	Selection from local germplasm	Andhra Pradesh	112	26.2
V25	GPU-58	GPU-26 × Sel-14	Bangalore, Karnatak	122	26
V26	AKP-2	Selection from Anakapalle local	Andhra Pradesh	90	20
V27	Indaf-5	Cauveri × IE-927	Madhya Pradesh	108	30
V28	AKP-7	Pure line selection from a local variety	Andhra Pradesh	115	17.0
V29	OEB-52	CO-12 × CO-13	Orissa	124	27.6
V30	OEB-82	CO-13 × PR-202	Orissa	123	26.0

DNA extraction

DNA was extracted from fresh leaves collected from 7-day-old seedlings raised in nutrient culture using the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). Approx. 20 mg of fresh leaves were ground to powder in liquid nitrogen using a mortar and pestle. The ground powder was transferred to a 50 ml falcon tube with 10 ml of CTAB buffer [2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris (tris(hydroxymethyl) aminomethane)-HCl, pH 8.0, and 0.2% (v/v) β-mercaptoethanol]. The homogenate was incubated at 60 °C for 2 h, extracted with an equal volume of chloroform/isoamyl alcohol (24:1 v/v) and centrifuged at $10{,}000 \times g$ for 20 min (Kubata KR-2000 C, Rotor-RA-3R, Tokyo, Japan). DNA was precipitated from the aqueous phase by mixing with an equal volume of isopropanol. After centrifugation at $10{,}000 \times g$ for 10 min, the DNA pellet was washed with 70% (v/v) ethanol, air-dried and resuspended in TE (10 mM Tris-HCl, pH 8.0, and 0.1 mM EDTA) buffer. DNA quantifications were performed by visualizing under UV light, after electrophoresis on 0.8% (w/v) agarose gel. The resuspended DNA was then diluted in TE buffer to 5 μg/μl concentration for use in polymerase chain reaction (PCR).

Primer screening for RAPD and ISSR analysis

Forty decamer primers, corresponding to kits A, B, D, and N from Operon Technologies (Alameda, California, USA) were initially screened using thirty varieties of finger millet to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products within the species/varieties. To ensure reproducibility, the primers generating no, weak, or complex patterns were discarded.

PCR amplifications for RAPD

Polymerase chain reactions with a single primer were carried out in a final volume of 25 μl containing 20 ng template DNA, 100 μM of each deoxyribonucleotide triphosphate, 20 ng of decanucleotide primer (M/S Operon Technologies), 1.5 mM $MgCl_2$, 1 × Taq buffer [10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.001% gelatin], and 0.5 U Taq DNA polymerase (M/S Bangalore Genei). Amplification was performed in a PTC-100 thermal cycler (M J Research Inc., Watertown, MA, USA) programmed for a preliminary 2 min denaturation step at 94 °C, followed by 40 cycles of denaturation at 94 °C for 20 s, annealing at 38 °C for 30 s and extension at 72 °C for 1 min, finally at 72 °C for 10 min for RAPD amplification. Amplification products were separated alongside a molecular weight marker (1.0 kb plus ladder, M/S Bangalore Genei) by 1.2% agarose gel electrophoresis in 1 × TAE (Tris acetate EDTA) buffer stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through a Gel Doc System (Gel Doc. 2000, BioRad, California, USA) and the amplification product sizes were evaluated using the software Quantity one (BioRad).

Data analysis

Data were recorded as presence (1) or absence (0) of band products from the photographic examination. Each amplification fragment was named by the source of the primer, 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 index was estimated using the Dice coefficient of similarity (Nei and Li, 1979). The average of similarity matrices was used to generate a tree by UPGMA (unweighted pair-group method arithmetic average) using NTSYS-PC, version 2.0 (Rohlf, 1995).

Results and Discussion

The present investigation offers an optimization of primer screening for the evaluation of genetic relationships among 30 genotypes of finger millet through RAPD markers. The genotype Dibyasinha was used for screening primers (derived from the series A, C, D and N) obtained from different series for amplification using polymerase chain reactions. The results showed that N- and C-series primers produced relatively more amplification fragments compared to A- and D-series decamer primers. The amplification generated by primers OPN-15, OPN-16 and OPA-13 produced a maximum number of DNA fragments; the size of the DNA fragments ranged from 300 to 3000 base pairs. Primer OPN-16 amplified 13 fragments, whereas OPA-13 produced 12 bands in the genotype Dibyasinha. It was also noted that some decamer primers did not show any amplification by using the genotype Dibyasinha. The twenty deca-

Name of primer	Sequence of the primer	Total number of amplification fragments	Number of polymorphic fragments
OPA-04	5′-AATCGGGCTG-3′	07	07
OPA-13	5′-CAGCACCCAC-3′	12	10
OPC-05	5′-GATGACCGCC-3′	07	07
OPC-08	5′-TGGACCGGTG-3′	08	08
OPC-12	5′-TGTCATCCCC-3′	10	10
OPC-18	5′-TGAGTGGGTG-3′	08	07
OPC-04	5′-CCGCATCTAC-3′	09	09
OPD-08	5′-GTGTGCCCCA-3′	11	11
OPN-03	5′-GGTACTCCCC-3′	10	10
OPN-08	5′-ACCTCAGCTC-3′	08	08
OPN-15	5′-CAGCGACTGT-3′	11	11
OPN-16	5′-AAGCGACCTG-3′	13	12
OPN-20	5′-GGTGCTCCGT-3′	10	10

Table II. Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected RAPD primers in 30 genotypes of *Eleusine coracana*.

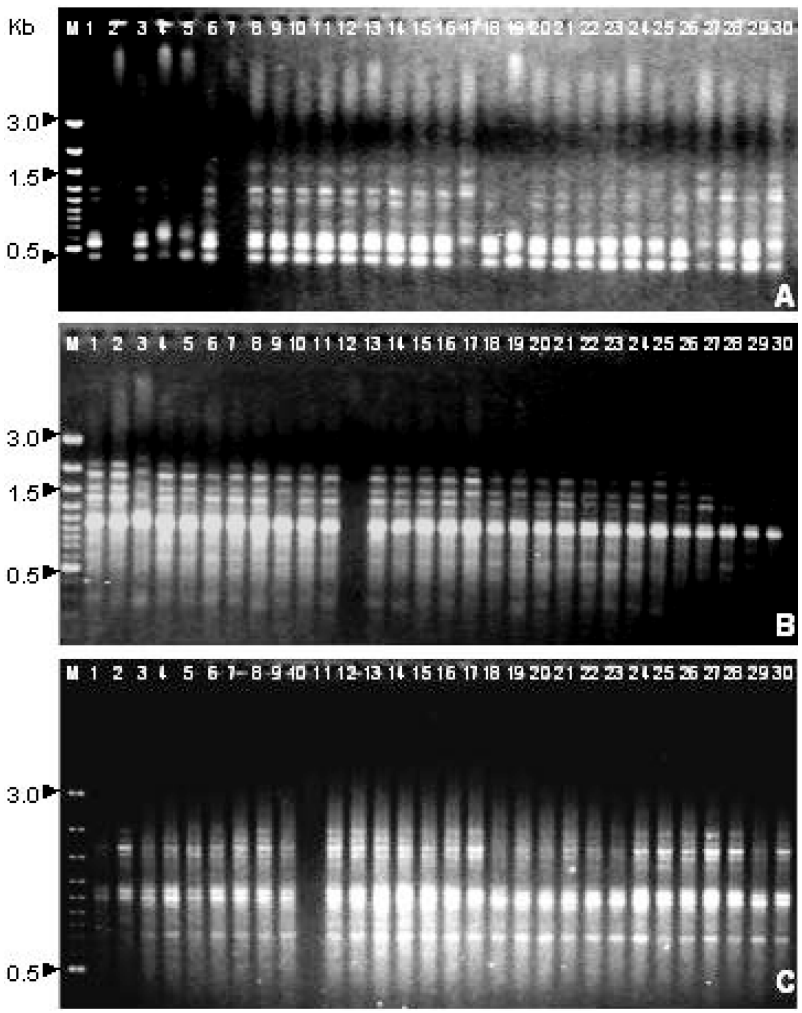

Fig. 1. RAPD patterns of 30 genotypes of *Eleusine coracana* generated by the primers OPC-18 (A), OPN-16 (B) and OPA-13 (C). M, molecular weight ladder (kb); 1–30, different genotypes of finger millet.

mer primers produced good amplification of RAPD fragments. Among the twenty primers, thirteen primers were selected to analyze the genetic relationships among the 30 genotypes of *Eleusine coracana* through RAPD markers. The reproducibility of the amplification product was tested with three independent extractions. Most of the amplification reactions were duplicated. Only bands that were consistently reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis. As a result, thirteen informative primers were selected and used to evaluate the degree of polymorphism within 30 genotypes of *Eleusine coracana*.

The maximum and minimum number of bands were produced by the primers OPN-16, OPA-13 and OPC-05, OPA-04, respectively (Table II). A total of 124 amplified fragments was scored across the 30 genotypes of *Eleusine coracana* for the selected primers, and used to estimate genetic relationships among themselves. The patterns of RAPD produced by the primers OPC-18, OPN-16 and OPA-13 are shown in Figs. 1A–C. The genetic variation through molecular markers has been highlighted in a number of cereal crops (Hilu, 1995; Salimath *et al*., 1995; Parani *et al*., 2001). Hilu (1995) reported that the pattern of genetic variation was closely correlated to geographic distribution. He also reported that genotypes of the subspecies *africana* did not group closely with those of the crop but showed higher affinities to *Eleusine indica*, reflecting the pattern of similarity revealed by an isozyme study. The present findings using RAPD markers show the close variation among the genotypes derived from the southern region and the distant variation among the genotypes collected from other regions of India. The similarity matrix was obtained after multivariate analysis using Nei and Li's coefficient and is presented in Table III. The similarity matrix was then used to construct a dendrogram with the UPGMA method (Fig. 2). The dendrogram shows two major clusters within 30 genotypes of *Eleusine coracana*. Between the two major clusters, one cluster has only one genotype (Dibyasinha) and the other major cluster divides into two minor clusters. The first minor cluster has only three genotypes, *i.e.* Chilika, Neelachal and OEB-56; all the three genotypes originated from Orissa have similar seed maturity duration. The second minor cluster has 26 genotypes and is again divided into two sub-minor clusters; the first sub-minor cluster having one genotype (*i.e.* VL-322) with 70% genetic similarity with the second sub-minor cluster and this second sub-minor cluster having 25 genotypes and is divided into two groups. The first group has one genotype and the second group has twenty-four genotypes. The second group again consists of two subgroups; one sub-group having 13 genotypes and the other sub-group having 11 genotypes. These two sub-groups have 78% similarity among themselves. Five genotypes (*i.e.* PR-202, RAU-8, VR-708, BM-107-2, SRS-2) are 100% similar among themselves. These five genotypes are harvested

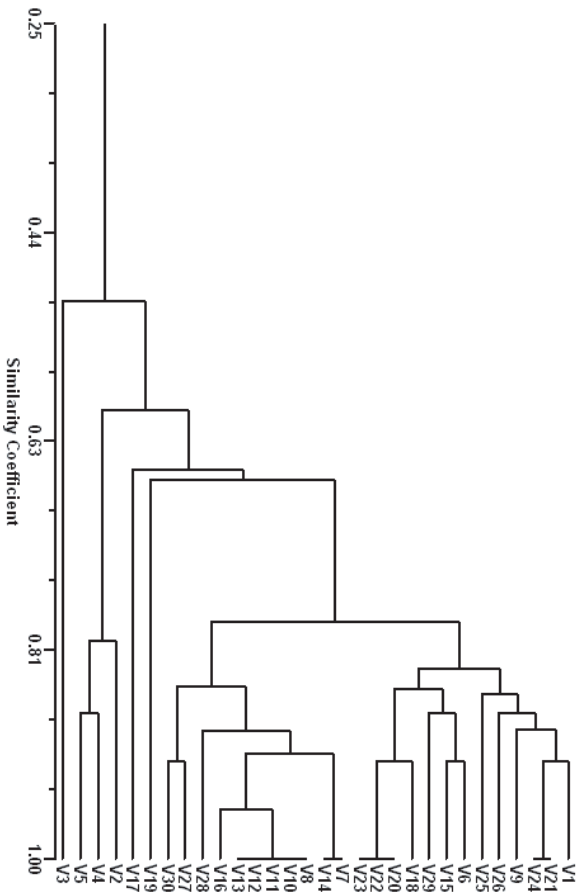

Fig. 2. Dendrogram of 30 genotypes of *Eleusine coracana* generated by UPGMA based on RAPD markers.

Table III. Similarity co-efficient of 30 varieties of finger millet by using RAPD primers.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29	V30
V1	1.00																													
V2	0.61	1.00																												
V3	0.52	0.13	1.00																											
V4	0.74	0.78	0.35	1.00																										
V5	0.70	0.83	0.30	0.87	1.00																									
V6	0.78	0.39	0.65	0.61	0.57	1.00																								
V7	0.87	0.57	0.48	0.70	0.57	0.83	1.00																							
V8	0.78	0.57	0.48	0.61	0.57	0.83	0.91	1.00																						
V9	0.83	0.43	0.61	0.65	0.61	0.87	0.87	0.87	1.00																					
V10	0.78	0.57	0.48	0.61	0.57	0.83	0.91	1.00	0.87	1.00																				
V11	0.78	0.57	0.48	0.61	0.57	0.83	0.91	1.00	0.87	1.00	1.00																			
V12	0.78	0.57	0.48	0.61	0.57	0.83	0.91	1.00	0.87	1.00	1.00	1.00																		
V13	0.78	0.57	0.48	0.61	0.57	0.83	0.91	1.00	0.87	1.00	1.00	1.00	1.00																	
V14	0.87	0.57	0.48	0.70	0.57	0.83	1.00	0.91	0.87	0.91	0.91	0.91	0.91	1.00																
V15	0.87	0.48	0.57	0.70	0.57	0.91	0.91	0.83	0.87	0.83	0.83	0.83	0.83	0.91	1.00															
V16	0.74	0.52	0.52	0.57	0.52	0.87	0.87	0.96	0.83	0.96	0.96	0.96	0.96	0.87	0.87	1.00														
V17	0.57	0.61	0.35	0.65	0.61	0.61	0.61	0.70	0.74	0.70	0.70	0.70	0.70	0.61	0.61	0.65	1.00													
V18	0.74	0.52	0.52	0.65	0.70	0.78	0.70	0.70	0.74	0.70	0.70	0.70	0.70	0.70	0.78	0.74	0.48	1.00												
V19	0.70	0.57	0.48	0.70	0.65	0.65	0.65	0.57	0.70	0.57	0.57	0.57	0.57	0.65	0.74	0.61	0.52	0.87	1.00											
V20	0.83	0.43	0.61	0.65	0.61	0.87	0.78	0.78	0.83	0.78	0.78	0.78	0.78	0.78	0.87	0.83	0.57	0.91	0.78	1.00										
V21	0.91	0.52	0.61	0.74	0.70	0.87	0.87	0.87	0.91	0.87	0.87	0.87	0.87	0.87	0.87	0.83	0.65	0.83	0.70	0.91	1.00									
V22	0.83	0.43	0.61	0.65	0.61	0.87	0.78	0.78	0.83	0.78	0.78	0.78	0.78	0.78	0.87	0.83	0.57	0.91	0.78	1.00	0.91	1.00								
V23	0.83	0.43	0.61	0.65	0.61	0.87	0.78	0.78	0.83	0.78	0.78	0.78	0.78	0.78	0.87	0.83	0.57	0.91	0.78	1.00	0.91	1.00	1.00							
V24	0.91	0.52	0.61	0.74	0.70	0.87	0.87	0.87	0.91	0.87	0.87	0.87	0.87	0.87	0.87	0.83	0.65	0.83	0.70	0.91	1.00	0.91	0.91	1.00						
V25	0.78	0.57	0.57	0.70	0.65	0.74	0.74	0.83	0.87	0.83	0.83	0.83	0.83	0.74	0.74	0.78	0.78	0.70	0.74	0.78	0.87	0.78	0.78	0.87	1.00					
V26	0.83	0.61	0.52	0.74	0.70	0.78	0.78	0.87	0.83	0.87	0.87	0.87	0.87	0.78	0.78	0.83	0.65	0.83	0.70	0.83	0.91	0.83	0.83	0.91	0.87	1.00				
V27	0.65	0.70	0.35	0.65	0.61	0.70	0.78	0.87	0.74	0.87	0.87	0.87	0.87	0.78	0.70	0.83	0.83	0.57	0.52	0.65	0.74	0.65	0.65	0.74	0.78	0.74	1.00			
V28	0.70	0.57	0.48	0.52	0.48	0.83	0.83	0.91	0.78	0.91	0.91	0.91	0.91	0.83	0.74	0.87	0.70	0.61	0.48	0.70	0.78	0.70	0.70	0.78	0.74	0.78	0.87	1.00		
V29	0.78	0.39	0.74	0.61	0.57	0.91	0.74	0.74	0.87	0.74	0.74	0.74	0.74	0.74	0.83	0.78	0.61	0.78	0.74	0.87	0.87	0.87	0.87	0.87	0.83	0.78	0.61	0.74	1.00	
V30	0.74	0.61	0.43	0.65	0.61	0.78	0.78	0.87	0.83	0.87	0.87	0.87	0.87	0.78	0.78	0.83	0.83	0.65	0.52	0.74	0.83	0.74	0.74	0.83	0.78	0.83	0.91	0.87	0.70	1.00

within 115 days from the day of seed sowing. The genotypes VR-849 and VR-768 have genetically 100% similarity with each other and they have originated from one location with similar maturity duration. These two genotypes are 90% similar with the genotype Bhairabi. The similarity matrix showed that the lowest and highest values were 0.13 and 1.0 among the 30 genotypes of *Eleusine coracana*. Parani *et al.* (2001) identified seven millet species using PCR-RFLP markers. They have achieved species-specific markers using two restriction enzyme combinations. There was no intraspecific variation among 20 accessions out of 119 accessions from seven small millet species. The present investigation showed that there is a narrow genetic variation among the genotypes derived from Northern as well as Southern India.

In conclusion, the result of this study indicates the efficiency and ease of using RAPD markers for investigating genetic relationships and identification of thirty genotypes of *Eleusine coracana*, a cereal crop. This information is helpful in breeding programs and is a major input into conservation biology in cereal crops.

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