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Molecular characterization of finger millet (*Eleusine coracana* L. Garten.) genotypes through RAPD markers

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Abstract
Thirty RAPD primers were utilized to identify the genetic variability of nineteen-finger millet genotypes using Random Amplified Polymorphic DNA (RAPD) molecular markers. A set of 15 RAPD markers that were among the primers used demonstrated polymorphism, and banding patterns were rated as 1 (present) or 0 (missing) in the datasheet. This data was then examined using SAHN clustering and Jaccard's similarity coefficient. The degree of polymorphism among the genotypes of finger millet was evaluated using the number of alleles, PIC value, and heterozygosity for each of the 15 RAPD markers. Fifteen polymorphic RAPD markers had 107 polymorphic loci in total. In 15 RAPD, the number of alleles generated per locus varied from 3 (OPD-13) to 13 (OPA-12). The average PIC value, according to RAPD analysis, was 0.33. In the marker analysis, the 19-finger millet genotypes were divided into two major clusters based on the data produced. Ten genotypes were displayed in RAPD cluster I, and nine genotypes in cluster II.

Keywords: RAPD, finger millet, polymorphism, molecular characterization

1. Introduction
97% of millets are produced and consumed in hot, arid parts of developing Asia and Africa, where they form the primary diet of impoverished farmers (McDonough *et al*., 2000) [1]. Millets are typically seen as a poor man's crop, despite being the sixth most widely cultivated grain in the world, behind rice, wheat, maize, barley, and sorghum (Dwivedi *et al.,* 2012) [2]. The Poaceae family includes the finger millet (*Eleusine coracana* (L.) Gaertn.) subspecies coracana. Also referred to as African millet, ragi, or nagli, finger millet is a crucial minor millet crop. Following sorghum and pearl millet in importance, it is the third most important millet globally. Right now, finger millet is the most important one economically. However, the tribal farmers who cultivate other small millet, such as little millet, barnyard millet, prose millet, foxtail millet, and kodo millet, also value them.

Both raw and processed finger millet are a good source of vitamins and dietary fibers. Ca, Mg, and K are among the higher minerals found in finger millet grains. Finger millet has a calcium concentration that is five to thirty times higher than other cereals. It also has an average protein content of 7.3% and a total amino acid composition of 44.7% of important amino acids (Nirgude *et al*., 2014) [3]. It is a healthy food that is advised for kids, those with diabetes, and people who are fat. (Ignacimuthu and Ceasar, 2012; Satish *et al*., 2016) [4, 5]. Additionally, it contains large concentrations of polyphenols (Chandrasekara 2011) [13] and amino acids (Bhatt *et al.,* 2016) [7] such as methionine, lysine, and tryptophan. Antioxidants and vital amino acids abound in grain protein.

India produced 1.1 million tons of agricultural products on 1.1 million hectares, with an average productivity of 1747 kg/hectare between 2016 and 2017 (Anon., 2022) [8]. Karnataka is the primary finger millet-growing state in India, with Uttarakhand, Maharashtra, Tamil Nadu, Andhra Pradesh, Gujarat, Orissa, Jharkhand, and Bihar following closely behind. There is an urgent need to genetically enhance finger millet yield because of the rising demand for the food crop and the shrinking land planted to it as a result of rival crops like maize and soybean.

Depending on the number of near ancestors and their phylogenetic position, molecular marker technology can be used to establish the distinctiveness and ranking of germplasm. The quick advancement of DNA marker technology offers great potential for raising the nutritional

content of conventionally grown grains and crops. The analysis and assessment of the finger millet genome have been greatly facilitated by recent advances in molecular biology, such as PCR, DNA sequencing, and data analysis technologies. PCR has emerged as a popular and practical technique for investigating the genome of rice, especially when assessing the genetic diversity of finger millet. It was predicted that during the next ten years, DNA markers will be the sole way to determine genetic variation in finger millet harvests at a lower cost and in less time.

2. Materials and Methods

In the current work, finger millet seeds were obtained from the hill millet research center, Navsari Agricultural University, Navsari, and a field trial was conducted at two locations *i.e*., Hill Millet Research Station Waghai, Gujarat 2) Wheat research station, Bardoli, Gujarat, India during summer 2022. The experiment was conducted in a factorial randomized block design (FRBD) with three replications, which included 19 genotypes *viz.*, PR-202, GN-8, GPU-45, GN-9, GPU-67, GNN-7, WN-581, GN-10, WN-569, WN-548, VL-352, WN-591, WN-550, WN-572, WN-566, WN-577, - 630, WN-593 and GNN-6 with three replications .

2.1 DNA extraction

Finger millet plants with 19 genotypes were harvested at the four-leaf stage for genomic DNA extraction, and DNA was obtained using the cetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle 1990). Using a spectrophotometer, the isolated genomic DNA was measured at 260 and 280 nm. The quantity and purity of the DNA were evaluated using agarose gel electrophoresis (0.8%).

2.2 PCR analysis and gel electrophoresis

A set of thirty RAPD primers was used. One sample's worth of genomic DNA (1 µl), master mix (5 µl), primer (2 µl), and sterile distilled water (2 µl) were used in the PCR process for RAPD markers. Using a BIO-RAD thermocycler machine, DNA replication using RAPD markers was carried out by polymerase chain reaction under the following conditions: 5 minutes of initial denaturation, 35 cycles of 30 seconds at 95 °C, 45 seconds for annealing, 60 seconds for extension, 10 minutes for final extension, and ∞ time at 4°C for a halt until samples were collected. The amplification products of the RAPD-PCR product were separated with a 1.8% agarose gel and a 100 bp DNA ladder.

2.3 Data analysis: For every genotype combination of the marker allele, the amplified output of the RAPD marker analyses was quantitatively graded as either present (1) or absent (0). The SAHN clustering function of PAST version 3.23 was used in conjunction with an agglomerative approach and the UPGMA method to conduct the cluster analysis. The SIMQUAL function estimated the similarity using Jaccard's similarity coefficient. Using the equation below, the polymorphism information content (PIC) was calculated. (Botstein *et al.* 1980) [10].

3. Results and Discussion

In a genomic investigation, molecular RAPD markers were employed to characterize genetic resources and evaluate the genetic variability in genotypes utilized in a breeding program. In the current study, the ability of RAPD primers to resolve heterogeneity amongst 19-finger millet genotypes was mostly dependent on diversity. Fifteen of the thirty RAPD markers that were utilized to create marker profiles were discovered to be polymorphic and appropriate for examination.

3.1 RAPD analysis

In an initial screening, the genetic diversity of the 19 finger millet genotypes was examined using 30 RAPD primers, of which 15 provided informative, polymorphic results that could be confirmed by agarose gel electrophoresis. Some of these products are displayed here (Figures 1–4). Polymorphic primers, the total number of loci, number of polymorphic loci, polymorphism percentage, and PIC value data are recorded in Table 1.

There we found a total of 103 loci in the study 86 (83.49%) of which were polymorphic, with PIC values ranging from 0.18 to 0.63 on average and values for several polymorphic loci from 2 to 13. Out of 15 primers, five primers (OPK-5, OPA-12, OPA-3, and OPA-6) primers exhibit 100% polymorphism. The marker OPK-07 had the lowest PIC value (0.18), whereas the marker OPA-06 had the greatest PIC value (0.63). Given the low genotypic variety of the 19-finger millet genotypes, the lower PIC value in this investigation may be explained. The nature of the materials under study can also be attributed to the high PIC value and large number of alleles per marker. When compared to the earlier research employing RAPD markers for genetic diversity in finger millet by Joshi *et al.* (2020) and Babu *et al.* (2007) [12], the average PIC value (0.33) in the current study is greater.

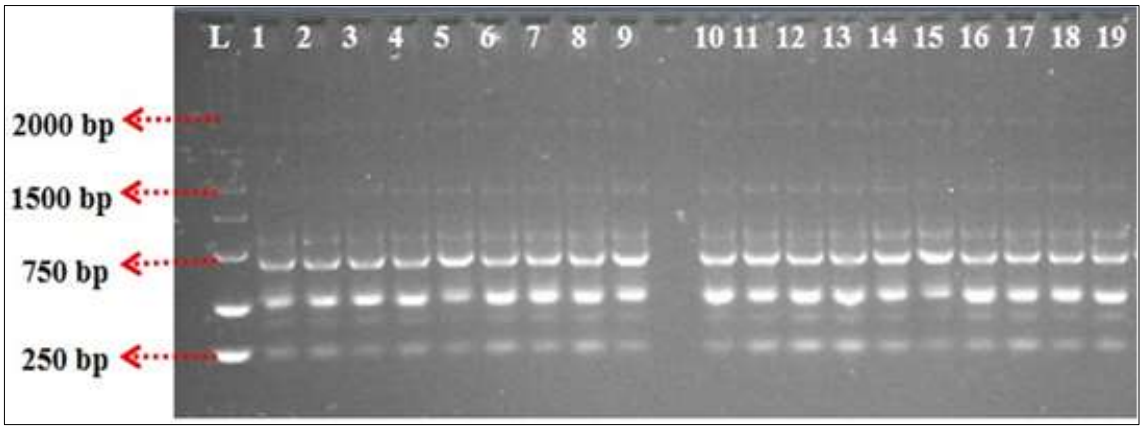

Fig 1: RAPD gel profiling of finger millet genotypes for OPK-5 primer

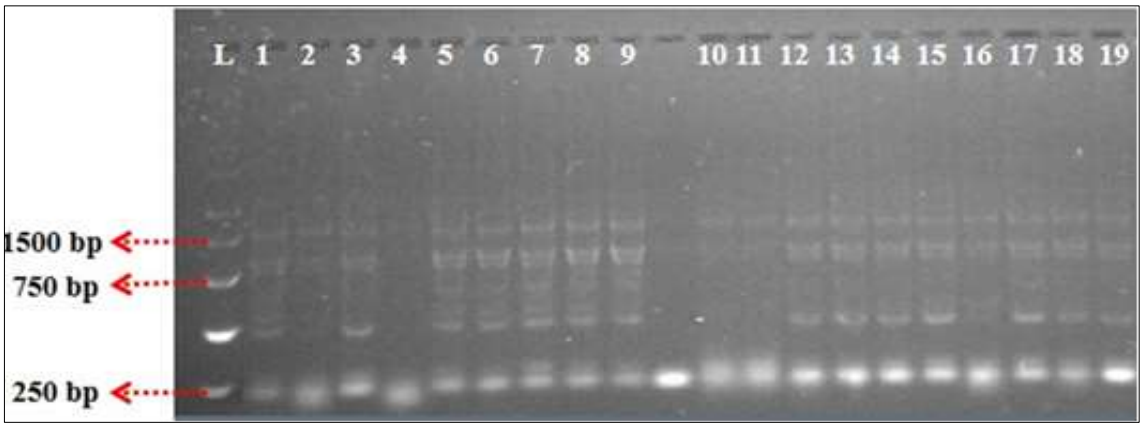

Fig 2: RAPD gel profiling of finger millet genotypes for OPA -2 primer

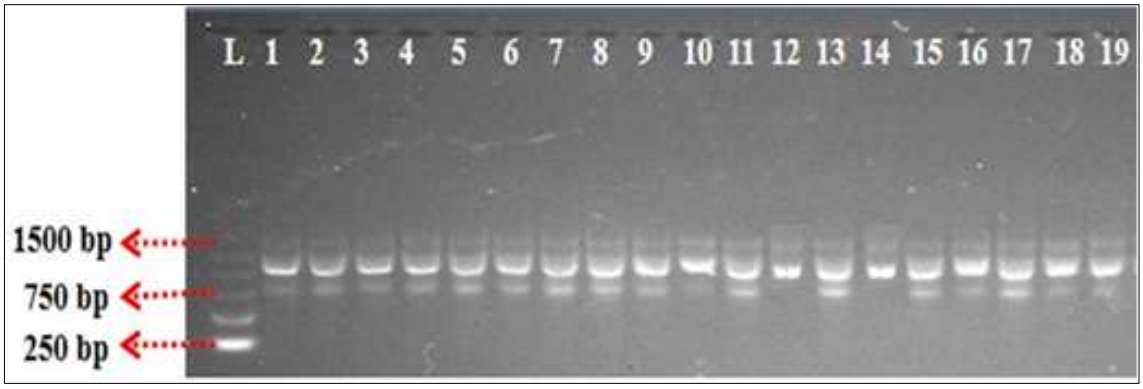

Fig 3: RAPD gel profiling for finger millet genotypes for OPA-3 primer

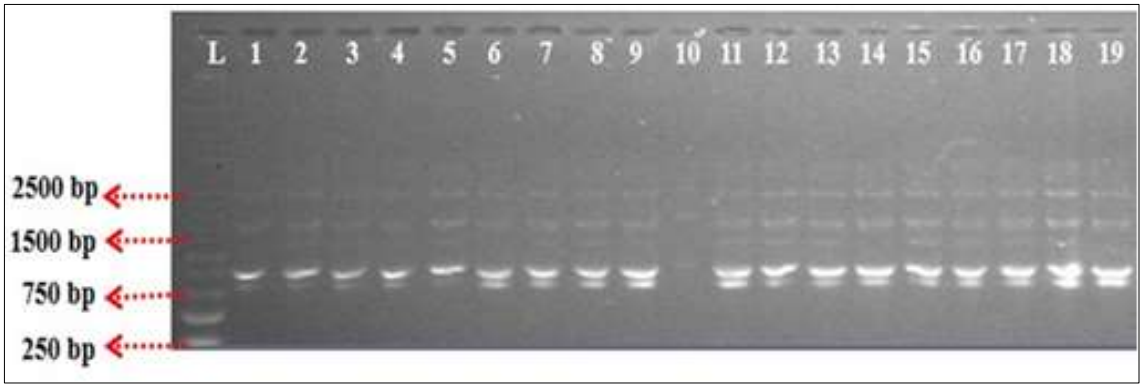

Fig 4: RAPD gel profiling of finger millet genotypes for OPA- -6 primer

Table 1: Polymorphism obtained with different RAPD primers in nineteen finger millet genotypes

Sr. No.	Primer	Total number of loci	Number of polymorphic loci	Percentage of polymorphism	PIC value
1.	OPK-7	6	3	50.00	0.18
2.	OPK-8	7	6	85.71	0.35
3.	OPK-5	6	6	100.00	0.24
4.	OPK - 6	10	7	70.00	0.28
5.	OPA-7	3	2	66.66	0.22
6.	OPD-7	5	2	40.00	0.34
7.	OPA-2	3	2	66.66	0.38
8.	OPK-2	4	3	75.00	0.30
9.	OPA-12	13	13	100.00	0.42
10.	OPA-10	9	8	88.88	0.31
11.	OPA-3	10	10	100.00	0.43
12.	OPA-6	10	10	100.00	0.63
13.	OPA-8	10	9	90.00	0.32
14.	OPD-11	4	3	75.00	0.31
15.	OPD-13	3	2	66.66	0.26
TOTAL		103	86	83.49	0.33

Cluster analysis and genetic diversity pattern based on RAPD marker

Using the UPGMA cluster seen in Figure 5, a dendrogram based on the similarity values produced by the RAPD was illustrated. Using the phylogenetic tree, 19 finger millet genotypes were separated into two major groups (A and B). From the primary cluster A, two sub-clusters were formed: A1 (6 genotypes) and A2 (4 genotypes). The genotypes WN-548, VL-352, WN-577, WN-593, GNN-6, and PR-202 were found in sub-cluster A1, while the genotypes WN-591, WN-550, WN-572, and WN-566 were found in sub-cluster A2. The genotypes of millet with nine fingers made up the second major cluster B. Two sub-clusters, B1 (three genotypes) and B2, were created by further separating key cluster B (6 genotypes). The three genotypes in subcluster B1 were GPU-67, GNN-7, and GN-10. Subcluster B2 consisted of six genotypes, namely GPU-45, WN-581, and WN-569.

The genotypes' genetic coefficients of similarity varied from 0.40 to 0.79. The genotypes GNN -6 and WN-593 had the greatest genetic distance (0.79), indicating that they differ greatly at the genomic level. This pair of genotypes can be used to expand the genetic background of different finger millet genotypes through biparental mapping populations and improvement initiatives. The genotypes GN-9 and WN-548 showed the lowest genetic distance (0.44).

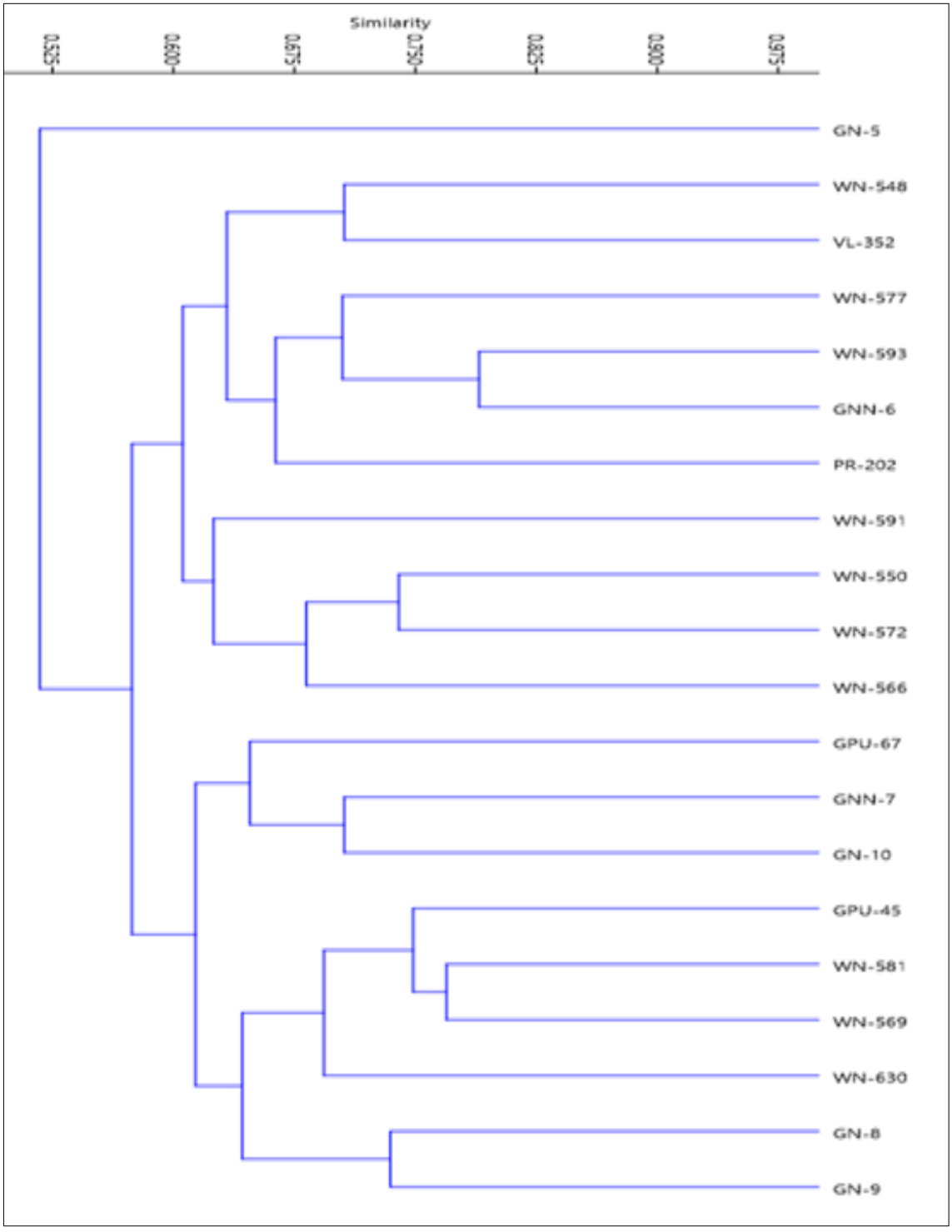

Fig 5: Dendrogram generated for nineteen finger millet genotypes using UPGMA cluster analysis based on RAPD marker

Table 2: Jaccard's similarity coefficient for 19 genotypes of finger millet based on RAPD molecular marker

	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1.00																	
2	0.53	1.00																
3	0.65	0.68																
4	0.55	0.73	1.00															
5	0.54	0.60	0.64	1.00														
6	0.60	0.56	0.58	0.69	1.00													
7	0.54	0.61	0.63	0.67	0.61	1.00												
8	0.62	0.58	0.69	0.61	0.71	0.62	1.00											
9	0.58	0.58	0.64	0.63	0.56	0.77	0.69	1.00										
10	0.60	0.47	0.44	0.47	0.51	0.54	0.53	0.54	1.00									
11	0.54	0.51	0.49	0.49	0.55	0.62	0.63	0.57	0.71	1.00								
12	0.53	0.50	0.48	0.58	0.56	0.61	0.59	0.54	0.53	0.69	1.00							
13	0.58	0.58	0.60	0.57	0.51	0.59	0.70	0.64	0.55	0.62	0.60	1.00						
14	0.65	0.51	0.55	0.60	0.58	0.56	0.68	0.66	0.60	0.62	0.70	0.74	1.00					
15	0.56	0.58	0.53	0.61	0.61	0.58	0.58	0.57	0.61	0.61	0.57	0.68	0.68	1.00				
16	0.64	0.47	0.55	0.50	0.68	0.64	0.67	0.60	0.60	0.65	0.53	0.63	0.64	0.62	1.00			
17	0.61	0.61	0.65	0.62	0.56	0.69	0.59	0.70	0.56	0.56	0.51	0.60	0.59	0.58	0.63	1.00		
18	0.69	0.51	0.52	0.50	0.58	0.63	0.63	0.65	0.68	0.66	0.56	0.62	0.65	0.57	0.72	0.71	1.00	
19	0.67	0.47	0.52	0.52	0.64	0.59	0.63	0.60	0.66	0.68	0.62	0.64	0.69	0.61	0.69	0.60	0.79	1.00

Genotypes Name: PR-202, GN-8, GPU-45, GN-9, GPU-67, GNN-7, WN-581, GN-10, WN-569, WN-548, VL-352, WN-591, WN-550, WN-572, WN-566, WN-577, WN630, WN-593 and GNN-6

Conclusion

Genetic variety should be a fundamental component of any breeding effort used in crop enhancement initiatives. Based on a molecular diversity study utilizing RAPD markers, genotypes GNN -6 and WN-593 were shown to have the maximum genetic distance in the main cluster among the 19-finger millet genotypes. For breeding operations, these can serve as genetically distant parents. Linkage analysis was aided by the PIC values, which identified the polymorphism between genotypes for a marker locus and the most useful RAPD markers, which are OPK-05, OPA-02, OPA-03, OPA-6, and OPR-05. This experiment's use of RAPD markers demonstrates their value in assessing the genetic diversity of finger millet genotypes.

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