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Genetic Diversity Analysis among Finger Millet Genotypes using Simple Sequence Repeat Markers

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ABSTRACT

Finger millet is a nutritionally rich, climate-resilient cereal crop with significant genetic variability. Understanding its genetic diversity is essential for effective conservation, improvement, and breeding programs. In this study, 4 simple sequence repeat markers were used to assess the genetic diversity among 7 finger millet genotypes. Microsatellites based genetic similarity between IE2051 and RAU-8 (0.7500) and IE2253 and IE2252 (0.7500) was found to be the maximum amongst pair-wise combinations of the entries. Cluster analysis using the UPGMA method grouped the 7 genotypes into 2 major clusters, and further into subclusters, suggesting clear genetic relationships among finger millet genotypes. The findings of this study highlight the effectiveness of SSR markers in detecting genetic variability and identifying genetically diverse genotypes. This result can be utilized for breeding strategies, germplasm conservation, and genetic enhancement of finger millets. SSR markers effectively revealed significant genetic diversity among finger millet genotypes. SSR markers proved to be highly informative and effective for detecting polymorphism among finger millet genotypes. The result will contribute to the breeding program for improving finger millet genotypes.

Keywords: Finger millet, SSR, DNA isolation, Polymorphism

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INTRODUCTION

Finger millet (Eleusine coracana (L.) Gaertn.), commonly known as ragi, is an important small-grain cereal crop cultivated predominantly in arid and semi-arid regions of Africa and South Asia. Finger millet, a highly self-pollinating crop, is a good source of micronutrients like iron and zinc (Dida et al., 2008). The grain contains 70-76% of carbohydrates, 7-14% crude protein, and is particularly rich in methionine, iron, and calcium (Babu et al., 2012). Valued for its exceptional nutritional profile, particularly its high calcium, dietary fiber, and essential amino acid content, finger millet plays a vital role in ensuring food and nutritional security, especially among resource-poor farming communities. Despite of resilience of millets to adverse environmental conditions (Swapnil et al., 2024) such as drought and poor soils, the productivity of finger millet remains relatively low, partly due to limited genetic improvement efforts and a narrow genetic base in cultivated varieties (Kumari and Singh, 2018; Kumari et al., 2020).

Genetic diversity analysis is fundamental for crop improvement, as it provides insights into the extent of variability present within and between genotypes, enabling the identification of potential parental lines for breeding programs (Raturi et al., 2024). There are very few reports available on the assessment of genetic diversity among finger millet at the national and international level (Babu et al., 2025). Among molecular markers, Simple Sequence Repeats (SSRs), also known as microsatellites, are highly preferred for diversity studies due to their abundance, co-dominant inheritance, high reproducibility, and ability to detect polymorphism even among closely related genotypes. SSR markers are particularly useful for characterizing germplasm collections, assessing population structure, and guiding strategies for conservation and utilization of genetic resources. In finger millet, the application of SSR markers can help unravel the genetic relationships among diverse genotypes, reveal patterns of genetic differentiation, and assist in selecting promising lines for hybridization. Such molecular-level insights, combined with morphological and agronomic evaluation, are crucial for broadening the genetic base and accelerating varietal development. The present study aims to assess the genetic diversity among finger millet genotypes using SSR markers, thereby providing a scientific basis for effective germplasm management, breeding, and conservation strategies.

MATERIALS AND METHODS

Experimental materials were comprised of seven genotypes (Table 1), namely, RAU-8, IE2051, IE2104, IE2235, IE2252, IE2253, and IE2293 of finger millet. Seeds of each of these genotypes were planted in pot containers filled with soil. The immature leaves from 15 days old seedlings were collected from each genotype and used for DNA extraction in the Department of Plant Biotechnology, College of Agricultural Biotechnology, Bihar Agricultural University, Sabour,

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Bhagalpur, Bihar, India. The extraction buffer was prepared by using 100 mM Tris-HCl (pH 8.0), 20 mM Na-EDTA salt (pH 8.0), 2 M NaCl, 3% (w/v) CTAB, 2% (w/v) PVP, and 1% β-Mercaptoethanol. Total genomic DNA was isolated by using the CTAB method (Doyle and Doyle, 1990) with slight modifications (Fig.1). The isolated DNA samples were subjected to agarose gel electrophoresis to assess their quality. The appearance of a single, sharp band of high molecular weight without smearing indicated the extraction of a good quality DNA sample with less damage during the extraction process. DNA from these samples was used for amplification using SSR markers.

Using standard protocol of polymerase chain reaction adjusted to laboratory conditions, a panel of four microsatellite primer pairs (Table 2) based amplification of targeted genomic regions was carried out in 15 µl reaction mixture with the help of a thermal cycler (Biometra), The primer vials were centrifuged before and after the addition of 1X TE buffer to the vials and sub-stocks were prepared from the stock. The reaction mixture was prepared by a combination of 2.6 µl water (Protease and Nuclease Free), 3.0 µl 5X buffer, 1.5 µl MgCl2 (10 mM), 3.0 µl dNTPs (200 µM), 1.2 µl Primer F (5 µM), 1.2 µl Primer R (5 µM), 0.5 µl Taq DNA polymerase (1 unit) and 2.0 µl DNA sample. Annealing temperature for different primer pairs was kept approximately 55°C less than their melting temperature (Tm) and amplification was carried out with an initial denaturation for 4 min at 94°C followed by 35 cycles of denaturation for 1 min at 94°C, primer annealing for 1 min at 55°C and primer extension for 2 min at 72°C and subsequently final extension for 10 min at 72°C. The products generated by the amplification reaction were subjected to agarose (2%) gel electrophoresis at 120 V for one and half hours and then visualized and documented under gel documentation system. The size of the amplified product was determined in relation to the size of markers in 50 bp DNA ladder (Fermentas) with the help of AlphaView gel reader. The position of the amplified products corresponded to the location of the bands along y axis (ranging from 0 to 1030). The Rf value for each band was determined assuming the location of the well as the initial position (Rf=0) and the position of migrated dye as the final position (Rf=1) in a frame of reference. A binary data matrix was generated by scoring for the presence and absence of bands in all the entries. The data matrix was subjected to cluster analysis. Similarity coefficients (Dice, 1945) were computed for pair-wise comparisons based on the proportions of shared bands produced by the

$$Similarity\ coefficient = \frac{2a}{(2a + b + c)}$$

Where, = Number of bands shared between Jth and Kth genotypes; b= Number of bands present in J^{th} genotype but absent in Kth genotype; and c= Number of bands absent in Jth genotype but present in Kth genotype.

Hierarchical cluster analysis was performed using the data on similarity coefficients. Tree building in the cluster analysis involved sequential agglomerative hierarchical nonoverlapping (SAHN) clustering. The dendrogram based on similarity indices was obtained by the unweighted pair-group method using arithmetic mean (UPGMA). Analysis was performed with the help of NTSYS-pc software (Rohlf, 1997). The nature of differentiation and divergence at the molecular level was examined by identifying the clusters. Principal coordinate analysis of the microsatellite primers dependent genetic profiles was conducted and compared with the results obtained from cluster analysis and neighbour joining tree.

Table 1: List of 7 finger millet genotypes used in the study

S. No.	Name of genotypes	Source
1	RAU-8	ICRISAT, India
2	IE 2051	ICRISAT, India
3	IE 2104	ICAR, India
4	IE 2235	ICRISAT, Africa
5	IE 2252	ICRISAT, India
6	IE 2253	IARI, India
7	IE 2684	ICRISAT, Malawai

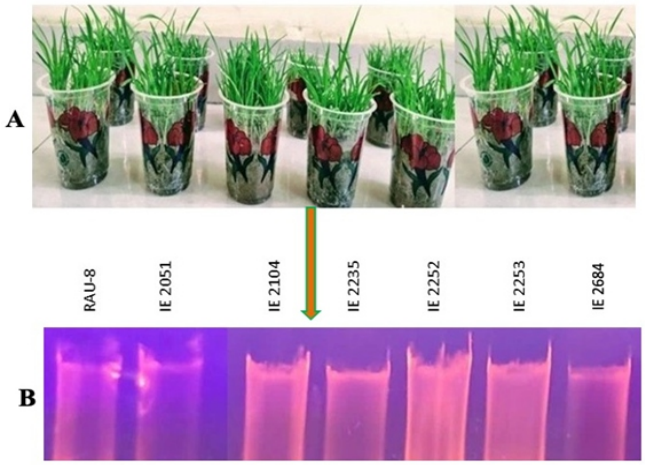

Fig. 1: Finger millet genotypes grown in pots for DNA isolation B: DNA extracted from seedling of finger millet genotypes

Table 2: List of four SSR primer pairs used for the amplification of genomic template extracted from 7 finger millet genotypes

S. No.	Primer	Sequence (5'->3')	Annealing temp. (0C)
1	UGEP03	CCACGAGGCCATACTGAATAG	55
		GATGGCCACTAGGGATGTTG	
2	UGEP05	TGTACACAACACCACACTGATG	55
		TTGTTTGGACGTTGGATGTG	
3	UGEP24	GCCTTTTGATTGTTCAACTCG	55
		CGTGATCCCTCTCCTCTCTG	
4	UGEP53	TGCCACAACTGTCAACAAAAG	55
		CCTCGATGGCCATTATCAAG	

RESULTS AND DISCUSSION

Computational analysis of the molecular size of different bands reflected different levels of molecular genetic polymorphism amongst the seven finger millet genotypes (Fig. 2). Allelic variants were generated by some of the primer pairs as a result of variation in the length of microsatellites among the finger millet genotypes (Palanga et al., 2016), while some generated only a few alleles. The differences in the size of amplified products, as recognized in the present study, reflect the variability in the length of the simple sequence repeats at a microsatellite site, which arises as a result of differences in the number of repeats existing in different entries and revealed by different primer pairs at the microsatellite locus in question. Therefore, the results of the molecular analysis clearly indicated the existence of ample genetic variability in terms of differences in the number of repeats at the primer specific microsatellite locus in the different finger millet genotypes under evaluation.

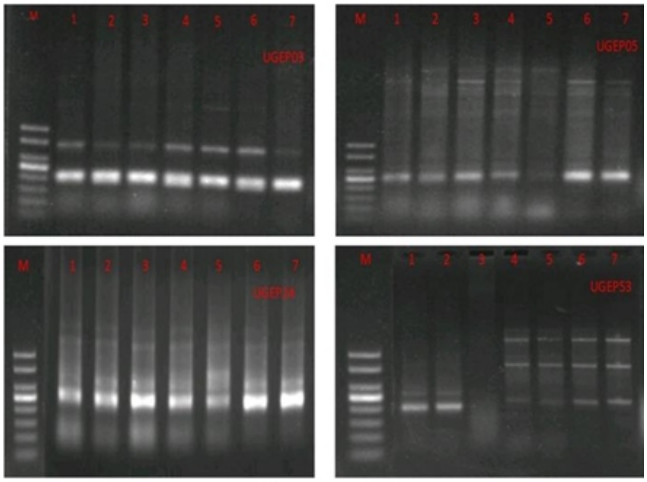

Fig. 2: Amplification pattern of the targeted genomic region in seven genotypes of finger millet obtained with the primer pairs UGEP 03, UGEP 05, UGEP 24, and UGEP 53

Genetic Diversity Analysis at Early Seedling Stage of Finger Millet Genotypes

Cluster analysis was performed using the data on similarity coefficients for four primer pairs. The method used for tree building in the cluster analysis involved SHAN. The dendrogram based on similarity indices was obtained by UPGMA. Analysis was performed with the help of NTSYS-pc software (Rohlf, 1997). The nature and extent of diversity between the finger millet genotypes under evaluation in the present investigation were assessed by identifying the clusters at appropriate phenon levels. Microsatellite-based genetic similarity (Table 3) between IE2051 and RAU-8 (0.7500) and IE2253 and IE2252 (0.7500) was found to be the maximum amongst pair-wise combinations of the entries. The magnitude of similarity coefficient between IE2252 and RAU-8, IE2252 and IE2051, IE2252 and IE2104, IE2253 and RAU-8, IE2253 and IE2051, IE2253 and IE2104, IE2684 and RAU-8, IE2684 and IE2051, IE2684 and IE2104 was equal to zero. Apparently, therefore, the microsatellite markers based molecular profiles did not exhibit any similarity between the entries involved in these nine pair-wise combinations. Overall, the results revealed enormous diversity at the molecular level (Rabbani et al., 2010; Behera et al., 2012; Pachauri et al., 2013; Saheewala et al., 2014; Palanga et al., 2016; Krupa et al., 2017) amongst the entries evaluated in the present analysis.

Based on the dendrogram, the finger millet genotypes were categorized broadly into two groups (Fig. 3), which were then further categorized into clusters and sub-clusters. The first multi-genotypic group consisted of four genetically improved finger millet genotypes, namely, RAU-8, IE2051, IE2104, and IE2235. While the second multi-genotypic group consisted of three entries, namely, IE2252, IE2253, and IE2684. Inferences derived from the results of similarity coefficients based hierarchical classification were completely corroborated by the principal coordinate analysis (Fig. 4) based on two two-dimensional plots of four microsatellite primer pairs dependent genetic profiles from finger millet genotypes. Thus, principal coordinate analysis based on two-dimensional plots of four microsatellite primers dependent genetic profiles revealed the spatial distribution pattern of entries along the two principal axes.

Table 3: Estimates of four SSR primer pairs based Dice similarity coefficients among seven finger millet genotypes used in the present study

	RAU-8	IE2051	IE2104	IE2235	IE2252	IE2253	IE2684
RAU-8	1.0000						
IE2051	0.7500	1.0000					
IE2104	0.2500	0.5000	1.0000				
IE2235	0.2500	0.5000	0.5000	1.0000			
IE2252	0.0000	0.0000	0.0000	0.5000	1.0000		
IE2253	0.0000	0.0000	0.0000	0.5000	0.7500	1.0000	
IE2684	0.0000	0.0000	0.0000	0.2500	0.2500	0.5000	1.0000

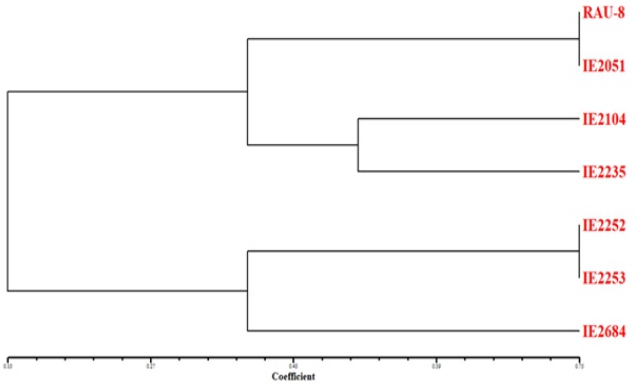

Fig. 2: Dendrogram based on average dice similarity coefficient for four SSR primer pairs among seven finger millet genotypes

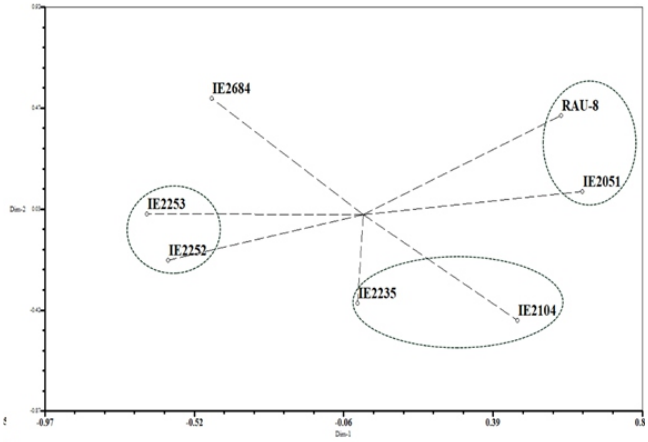

Fig. 2: Spatial two dimensional distribution of 4 SSR primers based genetic profile of 7 finger millet genotypes

CONCLUSION

A total of 7 finger millet genotypes were observed for 4 primer pairs. Microsatellite-based genetic similarity between IE2051 and RAU-8 (0.7500) and IE2253 and IE2252 (0.7500) was found to be the maximum amongst pair-wise combinations of the entries. Cluster analysis using the UPGMA method grouped the 7 genotypes into 2 major clusters and further subclusters, suggesting clear genetic relationships among finer millet genotypes. Results obtained from the similarity coefficient-based dendrogram among 7 finger millet genotypes were completely supported by PCA. SSR markers effectively revealed significant genetic diversity among finger millet genotypes. SSR markers proved to be highly informative and effective for detecting polymorphism among finger millet genotypes. The results will contribute to the breeding program for improving finger millet genotypes.

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