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Diversity Assessment in Cultivated Finger Millet [*Eleusine coracana* (L.) Gaertn.] Genotypes using SSR Markers

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ABSTRACT: Finger millet [*Eleusine coracana* (L.) Gaertn.] is one of the most important minor millets available in the world, and is cultivated mostly in harsh tropical weather of Africa and South Asia. It is a nutri-cereal, rich in protein and calcium with allied health benefits and is a potent crop for food security, though only negligible improvement has been observed for the crop so far because it was not a mainstream crop but now a days it is a better crop to cultivate in arid and semi-arid locations due to its capacity to flourish in marginal areas with little irrigation and poor soil fertility. Assessment of genetic diversity is a basic requirement for crop improvement for which simple sequence repeat (SSR) molecular markers are the most feasible technologies available in terms of specificity and cost-effectiveness. The present investigation was aimed to assess molecular divergence in 25 finger millet accessions through 19SSR markers, of which eight showed polymorphism. A total of 25 SSR amplicons (72 to 291 base pairs) was observed. Major allele frequency per marker (MAF), effective allele frequency per marker (A_e), heterozygosity (H_e) and Polymorphic Information Content (PIC) had an average of 0.48, 2.91, 0.63 and 0.61 respectively. Six markers had higher H_e and PIC hence could be exploited for agro-economic trait association studies. It was depicted that number of genotypes responding per marker (GR) had an inverse relation with A_e, whereas, A_e had a positive relation with H_e and PICbuta negative relation with MAF. Euclidean distance method revealed considerable divergence between genotypes with degree of dissimilarity ranging from 1.751 to 4.406 indicating that the accessions might be utilized for crop improvement in finger millet. Dendrogram revealed two distinct clusters, white seeded cultivars formed one cluster and the rest formed another indicating involvement of distinct gene expression for grain colour. The diverse accessions might be utilized for crop improvement.

Keywords: Clustering, Diversity, Finger millet, SSR, PIC, Euclidean distance.

INTRODUCTION

Finger millet [*Eleusine coracana* (L.) Gaertn.] is an annual diploid (2n=4x=36) herb belonging to Poaceae family. It is widely grown as a cereal crop in arid and semiarid areas of Africa and South Asia where calcium deficiency and anemia are widespread within human population. In India it is known as 'Ragi' and is the principal food grain to rural population in Western and Southern India. It is considered as a nutri-cereal because of its higher protein (6–13%) and calcium (0.3–0.4%) content (Panwar *et al.*, 2010) than rice, corn or sorghum along with good amount of fiber and minerals.

Despite finger millet's potential for providing nutrition and food security, negligible scientific inputs are observed on improvement of this crop. A significant portion of the finger millet germplasm collections are found in field and *in vitro* genebanks. In gene banks throughout the world as of 2010, 35382 finger millet accessions were preserved. The International Crops Research Institute for the Semi-Arid (ICRISAT) and the National Bureau of Plant Genetic Resources (India) genebanks, respectively, accounted for 26.9% and 16.8% of the world's collections. The lack of data has prevented finger millet research and breeding programmes from using genebank accessions in a systematic manner (FAO, 2010; Mbinda and Masaki, 2021). Assessment of genetic diversity has been a major goal for improvement of any and every crop (Laurentin, 2009; Dong *et al.*, 2014; Yaman *et al.*, 2014; Zhang *et al.*, 2014). Moreover, modern technologies offer precise and faster assessment of existing genetic diversity and its utilization in manipulating desirable traits (Sood *et al.*, 2016) for a better economic outcome. SSRs are one of the most cost-effective DNA markers due to their abundance throughout genome, high level of polymorphism, locus-

specificity, multi-allelic and co-dominant nature and reproducibility (Powell *et al*., 1996). SSRs can be developed by constructing microsatellite enriched genomic libraries or *in silico* from genome sequences available in public databases. Present investigation on "Molecular diversity analysis in cultivated finger millet [*Eleusine coracana* (L.) Gaertn.] genotypes using SSR markers" was undertaken to assess molecular diversity in 25 finger millet genotypes thereby checking their potential for further exploitation in improvement of the crop.

MATERIALS AND METHODS

Plant material and DNA extraction. Experimental material was comprised of 25 genotypes (Table 1) collected from Department of Genetics and Plant breeding, B.A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India. Leaves were collected while the crop was at university experimental field (22.5359° N, 72.9749° E) and utilized for genomic DNA isolation through modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle 1987).

DNA quantification. Quality of isolated genomic DNA was assessed through agarose gel (1.0%) electrophoresis in 1X TBE buffer with Ethidium Bromide (EtBr) as fluorescent dye and DNA ladder of 20bp or 100bp (as per need) as standard molecular marker. On completion of run, genomic DNA was visualized by placing the Gel under UV light in gel documentation system (BIO RAD). Clear bands indicated pure genomic DNA while smears indicated protein and RNA contamination.

Quantity and quality of isolated genomic DNA was estimated further by spectrophotometer instrument (NanoDrop-1000, Software V.3.3.0) for a precise assessment. Concentrations of 1µl isolated DNA at $A_{260/280}$ were measured. Pure DNA has $A_{260/280}$ ratio around 1.8 but less than 2.0 in 1X TE buffer. DNA was quantified in ng per µl. Total 50ng per µl of working DNA solution was prepared from the known quantity of stock DNA solution.

SSR-PCR parameters and gel analysis. Isolated genomic DNA was amplified using 19 SSR primers (Table 2). The Polymerase Chain Reaction (PCR) reaction mixture consisted of a total volume of 15µl containing 2µl template DNA, 6µl PCR master mix (EmeraldAmp GT PCR Master Mix 2x), 1µl diluted primer and 6µl nuclease free water. PCR reaction conditions were, initial denaturation at 94°C for 5 minutes, followed by 35 cycles of (94°C for 45 seconds, ΔT°C (primer specific) for 45 seconds, 72°C for 45 seconds), and then a final extension at 72°C for 7 minutes. PCR reactions were carried in Applied Biosystem Thermocycler (Veriti 96 well thermal cycler). PCR products were separated on 2.5% agarose gel and DNA ladder (either 20 bp or 100 bp) were used as a standard molecular marker for band size reference depending on different SSR loci. Separated bands were visualized under UV Trans-illuminator and were photographed using Syngene snap-G-box.

Data analysis. The size of amplified bands (in nucleotide base pairs) for each microsatellite marker was determined by AlphaEaseFC 4.0(Genetic Technologies, Inc., Miami, FL, USA) software based on its migration relative to a size marker of specific molecular weight (20 bp or 100 bp DNA Ladder). Banding pattern was recorded in the form of 0-1 matrix which was analyzed further based on Euclidean dissimilarity coefficient using R 64× 3.6.1 software and a dendrogram was constructed. Number of effective allele/s per marker, heterozygosity, major allele frequency and Polymorphic Information Content were calculated from the following formulae:

Effective allele (A_e)

$$A_e = 1/\sum_{i=1}^{k} P_i$$

where, P_i= i^{th} allele frequency

Heterozygosity (H_e)

$$H_e = 1-\sum_{i=1}^{k} P_i$$

where, P_i= i^{th} allele frequency

Major allele frequency (MAF)

Major allele frequency= [(Number of genotypes having major allele)/
(Total number of genotypes)] × 100

Polymorphism information content (PIC)

$$PIC = 1-\left(\sum_{i=1}^{k} Pi^2\right)$$

where, P_i= i^{th} allele frequency

Correlation coefficient

The correlation coefficient between parameters was calculated according to Pearson, 1895.

$$r-\Sigma = \frac{(x-\bar{x})\,(y-\bar{y})}{\sqrt{\Sigma\,(x-\bar{X})^2}\ \sqrt{(y-y)^2}}$$

Where, $\bar{x}$ = mean of X variable
$\bar{y}$ = mean of Y variable

RESULTS AND DISCUSSION

DNA was isolated and purified from 25 finger millet genotypes and the DNA quality and quantity was checked through agarose gel electrophoresis and also by spectrophotometer. Strong absorbance at 280nm, resulting in a low $A_{260/280}$ ratio indicated presence of protein contaminants in sample, whereas a value more than 2.0 indicated presence of RNA. It was observed that all of the isolated DNA samples were pure having $A_{260/280}$ below 2.0 and above 1.8. No significant correlation was found between $A_{260/280}$ ratio and DNA concentration. 50ng per µl of working DNA solution was prepared from the known quantity of stock DNA solution.

Key to success of any breeding programs primarily depends on the extent and distribution of genetic variability in available germplasm. Phenological variation in finger millet has been reported by various researchers in India as well as in the world (Kumar *et al*. 2010; Babu and Agrawal 2014; Goswami *et al.*, 2015; Kumari *et al.*, 2018). However precise knowledge on true existing genetic variation can be

acquired through DNA markers as those are least responsive to environmental as well as physiological condition of an organism. Amongst DNA markers, though AFLP, RFLP, RAPD and ISSR (Dvorakova *et al*., 2016; Sood *et al.,* 2016) have been successfully deployed to reveal genetic diversity of finger millet, low reproducibility and technical complexity were found to be the major drawback in working with those marker systems. SSR in those aspects were found to be better in describing diversity in finger millet with greater efficiency (Gimode *et al*., 2016). Dida *et al* (2008), first developed genomic SSR markers for finger millet and offered its basic map followed by many workers adapting SSR technique for diversity analysis in finger millet (Panwar *et al*., 2010; Bharathi, 2011; Arya *et al*., 2013; Babu *et al*., 2014; De Villiers *et al*., 2015; Ramakrishnan *et al.*, 2016).

In the present study, a total of 19 SSR primers were screened and only eight primers (Table 2) showed good amplification with polymorphism revealing presence of considerable variability among 25 finger millet genotypes. Result is summarized in Table 3.

Performance of SSR markers. Number of average alleles per primer was found to be 3.125 and the average alleles per genotypes was one which was similar to results of 0.84, 1.02 and 1.06 alleles per genotypes of finger millet respectively reported by Panwar *et al.* (2010); Arya *et al*. (2013); Ramakrishnan *et al.* (2016) using SSR markers. Allelic size ranged from (75 to 291) bp, which was similar to allelic size (33bp to 268 bp and 153bp to 261bp) found by Selvam *et al.* (2015); Deshpande *et al*. (2018) respectively.

Major allele frequency (MAF) of SSR markers ranged from 0.34 to 0.66 and mean major allele frequency was 0.48, whereas mean major allele frequency obtained by Ramakrishnan *et al.* (2016) was 0.92 and Gimode *et al.* (2016) obtained 0.62.

Effective allele (A_e) per SSR marker ranged from 1.79 to 4.53 with an average value of 2.91 per marker. Effective alleles per SSR reported by Lee *et al.* (2017) ranged from 1.01 to 3.98, with a mean of 1.75 alleles per SSR marker.

Ramakrishnan *et al.* (2016) observed lower heterozygosity (H_e) value ranging from (0.0 to 0.26) with an average of 0.11 in comparison to the present result, which offered higher heterozygosity range of (0.44 to 0.78) with an average 0.63, whereas mean heterozygosity observed by Lee *et al*. (2017) was 0.27.

PIC values of eight SSR markers in the present study ranged from 0.46 (UGEP05) to 0.76 (FMO2-14) with an average of 0.61, which was comparatively more than 0.22, as reported by Deshpande *et al.* (2018) among 47 finger millet varieties, 0.34 as reported by Nethra *et al.* (2014) and 0.551 as reported by Panwar *et al.* (2010). PIC depends on many factors such as breeding behavior of a species, genetic diversity in experimental collection, sensitivity of genotyping method and location of primer in genome used for study (Singh *et al.*, 2013).

It was found that FMO2-14 offered five alleles, allele size was (179 to 241) bp, with lowest MAF, A_e and H_e but highest PIC. UGEP24 exhibited three alleles between (180-211) bp and low MAF, but near average A_e, H_e and PIC. UGEP53 also offered three alleles of higher molecular weight (217-247) bp, highest MAF but low A_e, H_e and PIC (below average). UGEP67 too exhibited three alleles of (72-189) bp, low MAF, highest A_e and H_e and above average PIC.UGEP93 also offered three alleles of (161-209) bp and near average MAF and H_e but below average A_e and PIC. UGEP101 exhibited three alleles at higher range (213-254) bp, with near average (MAF, H_e and PIC) values for all parameters except A_e which was low.

It was also observed that genotypes, namely, WN-629, WWN-34 and WN-584 did not respond for five, four and two SSR markers respectively; whereas GN-3, PR-202, GNN-7, WWN-35, WN-522, WN-544, WN-564, WN-568, WN-602, WN-630 and WWN-37, each were not responsive for different single markers.

Correlation Study. A graph was prepared (Fig.1) to find relationship of allele size (bp) and genotypic response if any. But no such relationship was observed, genotypes responding for different alleles offered by different markers were found to be random. The trendlines of the two parameters confirmed the same. Graphical representation of number of alleles (A) per marker, A_e, number of genotypes responding per marker (GR) revealed that GR had an inverse relation with A_e but had a positive relationship with number of alleles, whereas (A) and A_e were inversely related (Fig. 2). Another graphical representation (Fig. 3) for MAF, A_e, H_e and PIC for each marker revealed that A_e had a positive relation with H_e and PIC, whereas a negative relation was observed with MAF. MAF was also inversely related with H_e and PIC. A small positivity was noted between H_e and PIC.

Correlation coefficient (r)among all the above mentioned parameters (Table 4) offered a clear picture. Number of alleles per marker (A) had positive correlation only with GR and high positive relation with PIC which is obvious, because more the number of alleles, more varied will be response by the genotypes for each allele. GR had negative correlation with every parameter measuring diversity which is also quite obvious as more genotypes responding for a specific marker offers less diversity for the population under study (uniform response – monomorphic nature). Hence expectedly, number of genotypes not responding for any marker (GNR) shared the opposite relationship. It exhibited positive correlations with A_e and H_e and PIC. A_e shared positive correlation with H_e as it is actually an indicator of existing variation (Nei, 1987; Karabag *et al.*, 2016). Considering the facts above, PIC was supposed to share positive correlation with A_e and H_e which is actually the case here. Though correlation was found to be positive and negative as expected, for some cases it appeared significant and, in some case, it was not, that may be due to small population size from restricted geographical area. Generally, either heterozygosity or PIC can be calculated for assessing diversity in a given population. Heterozygosity assesses divergence whereas PIC is useful for linkage map, but as in the case here, two parameters do not show significant positive relation, hence it is better to

calculate both the parameters. It can be concluded that number of effective alleles is a good indicator of divergence and more varied the genotypic response is, more diverse the population is.

Moreover, it is effective allele which leads to heterozygosity and polymorphism amongst genotypes establishing a positive relationship amongst these three parameters. Genotypes not responding (GNR) to one or more alleles for any specific marker factually contribute to number of effective allele and heterozygosity. So GNR must relate with these parameters in a positive way, which is actually found in the present study.

Genotype performance. For a more precise inference, all the alleles were grouped into four categories, namely, <100bp (4 alleles), (151-200) bp (8 alleles), (201-250) bp (10 alleles) and >250bp (3 alleles) and individual genotypes responding to each group of alleles was calculated and a graph was then prepared to observe the individual genotypic response for each allelic group (Fig. 4). All the genotypes responded for the group with highest number of alleles *i.e.* (201-250) bp as the group had 10 alleles to respond to, followed by (151-200) bp group. For the group (201-250) bp, VL-149 responded most with six alleles, but WN-629 responded lowest with only one allele. In case of other two groups, though <100 bp (4 alleles) group had a greater number of alleles as compared to group of allele size of >250 bp (3 alleles), individual genotypic response was found higher for the latter group. The response of genotypes was concentrated for the allelic groups (151-200) bp and (201-250) bp (Fig. 4), but fewer genotypes responding for alleles belonging to <100 bp and >250 bp contributed more to the variation observed.

Varieties recommended for all Gujarat conditions, namely, GN-2, GN-3, GN-8 responded similarly for <100 bp and > 250 bp categories, but cultivar for South Gujarat condition which was GNN-7, gave an altogether different response than the previous three.

Uniform response for different allelic groups was also noticed in three of five cultivars recommended for all India cultivation, which were GPU-28, GPU-45 and GPU-67 with only a minor variation (GPU-28). Unlike these three cultivars, the other two cultivars recommended for all India cultivation, namely, PR-202 and VL-149, which are also National checks, offered varied response to the allelic groups different from each other. Much varied response was observed for the cultivars specific from Waghai, Gujarat, though white seeded varieties offered more uniform response to specific allelic groups.

Phylogenetic analysis. Among the all pair-wise combinations of genotypes, Euclidean dissimilarity coefficients based on SSR markers ranged from 1.751 to 4.406 (Table 5, Fig. 5). Twenty-five finger millet genotypes were grouped into two distinct major clusters based on Euclidean distance. Two major clusters were further divided into two sub-clusters (Fig. 5). No specific correlation was observed between place of adaptation/ recommendation of genotypes and their clustering pattern which was also confirmed through dissimilarity index values.

Cluster A comprised of 12 genotypes divided into two sub-clusters. Sub-cluster A_1 was comprised of eight genotypes and sub-cluster A_2 was comprised of four genotypes. Genotypes of both the sub-clusters offered similar response to allelic groups, namely, (151-200) bp and (201-250) bp (Fig. 3). Among those, GN-8 and WWN-37 and also GNN-7 and WWN-35 appeared to be genetically close to each other as evident from least dissimilarity coefficient values of 1.751 for both the cases (Table 5) and similar allelic response (Fig. 4). Cluster B was comprised of 13 genotypes. Sub-cluster B_1 consisted five genotypes, where, GPU-45 and PR-202, and also WN-564 and WN-593 appeared close with each other as expected from their pattern of allelic response and dissimilarity coefficient of 1.751 and 2.674 respectively (Table 5, Fig. 4). Sub-cluster B_2 consisted of eight genotypes, where all those genotypes offered much varied allelic response (Fig. 4) for the four allelic groups. Among those, GPU-28 and WN-542, GN-3 and WN-522 and also WN-467 and WN-587 appeared to be genetically close to each other which was also confirmed through low dissimilarity coefficients of 2.859 for the first genotypic pair and least dissimilarity coefficient of 1.751 for the other two genotypic pairs mentioned (Table 5).

It was observed that sub-cluster A_1 comprised of cultivars from Gujarat only. Moreover, in this cluster all the white seeded Gujarat varieties came together defining their close genetic background. Other three sub-clusters, namely, A_2, B_1 and B_2, had cultivars from Gujarat as well as cultivars released at all India level, establishing their broader genetic base. PR-202 and VL-149 both are national checks, but appeared in different clusters which confirmed their genetic distance and defined the reason behind selecting both the varieties as national check because national level trials are conducted at various agro-ecological zones which require broader genetic base for stable and uniform performance. The dendrogram and the phylogenetic tree generated from the data did not show any correlation between place of development or recommendation of any genotypes with its clustering pattern which re-established the fact that due to continuous gene flow, naturally and artificially, geographical distribution of genotypes does not correlate much at molecular level except conservative genotypes like the white seeded cultivars in the present investigation which were grouped in sub-cluster A_1. Grouping of all the white seeded genotypes in a single cluster indicated that grain colour is controlled by distinct gene expression. The results of the study of Suryanarayana *et al.* (2014) indicated that in Finger millet the geographical diversity is not a dependable feature in estimating genetic diversity. Even the results of Kumar *et al.* (2010) showed that the geographical diversity was not related to the genetic diversity in Finger millet.

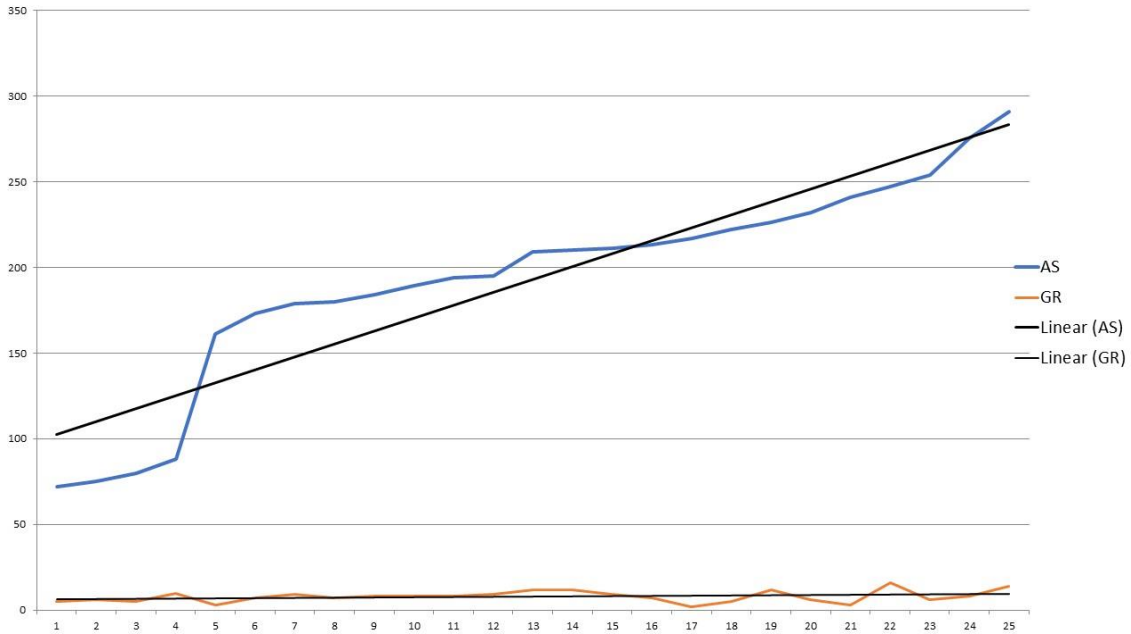
Table 1: List of genotypes used in study.

Sr. No.	Genotypes	Place of Release/Recommendation
1.	GN-2	All Gujarat
2.	GN-3	All Gujarat
3.	GN-8	All Gujarat
4.	GNN-7	South Gujarat
5.	GPU-28	Released variety at all India level
6.	GPU-45	Released variety at all India level
7.	GPU-67	Released variety at all India level
8.	PR-202	Released variety and National Check
9.	VL-149	Released variety and National Check
10.	WN-467	Waghai, Gujarat
11.	WN-522	Waghai, Gujarat
12.	WN-542	Waghai, Gujarat
13.	WN-544	Waghai, Gujarat
14.	WN-564	Waghai, Gujarat
15.	WN-568	Waghai, Gujarat
16.	WN-584	Waghai, Gujarat
17.	WN-587	Waghai, Gujarat
18.	WN-593	Waghai, Gujarat
19.	WN-602	Waghai, Gujarat
20.	WN-629	Waghai, Gujarat
21.	WN-630	Waghai, Gujarat
22.	WWN-32	Waghai, Gujarat (White seeded)
23.	WWN-34	Waghai, Gujarat (White seeded)
24.	WWN-35	Waghai, Gujarat (White seeded)
25.	WWN-37	Waghai, Gujarat (White seeded)

Table 2: List of polymorphic SSR primers.

Sr. No.	Primer	F/R	Sequence (5'->3')	AT (°C)
1.	UGEP03	F	CCACGAGGCCATACTGAATAG	59.6
		R	GATGGCCACTAGGGATGTTG	
2.	UGEP05	F	TGTACACAACACCACACTGATG	56.8
		R	TTGTTTGGACGTTGGATGTG	
3.	UGEP24	F	GCCTTTTGATTGTTCAACTCG	58.65
		R	CGTGATCCCTCTCCTCTCTG	
4.	UGEP53	F	TGCCACAACTGTCAACAAAAG	56.6
		R	CCTCGATGGCCATTATCAAG	
5.	UGEP67	F	CTCCTGATGCAAGCAAGGAC	59.4
		R	AGGTGCCGTAGTTTGTGCTC	
6.	UGEP93	F	TGGCCTCGTTAGGTGAAGTC	58.3
		R	AGCACCAAAACTCCCACAAC	
7.	UGEP101	F	GCTCACTTACCCATGGCTTC	58.3
		R	GAAATGTGGGGCACATAAGG	
8.	FMO2-14	F	ATATGGACTGACGACGCAAATA	54.7
		R	TGGAGAGATCAGAAGTAGACAAGG	

F: Forward; **R:** Reverse; **AT:** Annealing Temperature



AS = Allele size (bp); **GR** = Genotypes responded per specific allele size

Fig. 1. Genotypic response with respect to allele size.

Table 3: Detailed results observed for SSR markers used in molecular diversity study of finger millet.

Sr. No.	SSR primer	No. of alleles	Allele size (bp)	Name of genotypes		MAF	A_e	H_e	PIC
				Amplified in	Not amplified in				
1.	FMO2-14	5	179	GN-2, GN-3, GPU-67, WN-467, WN-522, WN-542, WN-564, WN-587, WN-630	WN-629	0.34	1.79	0.44	0.76
			194	GN-3, WN-467, WN-522, WN-544, WN-564, WN-584, WN-593, WWN-34					
			210	GN-8, GPU-28, GPU-45, PR-202, VL-149, WN-544, WN-568, WN-602, WWN-32, WWN-34, WWN-35, WWN-37					
			222	GN-2, GPU-28, GPU-45, GPU-67, PR-202					
			241	GNN-7, VL-149, WN-584					
2.	UGEP03	3	75	GN-8, GPU-67, VL-149, WN-544, WN-568, WN-584,	GNN-7, WN-629, WWN-34, WWN-35	0.47	3.88	0.74	0.63
			80	GN-3, GPU-28, WN-522, WN-542, WN- 602					
			88	GN-2, GPU-45, PR-202, WN-467, WN-564, WN-593, WN-630, WWN-32, WWN-37, WN-587					
3.	UGEP05	2	275	GN-3, GPU-28, PR-202, WN-467, WN-522, WN-542, WN-587, WN-630	WN-584, WN-629, WWN-34	0.63	2.40	0.58	0.46
			291	GN-2, GN-8, GNN-7, GPU-45, GPU-67, VL-149, WN-564, WN-568, WN-593, WN-602, WWN-32, WWN-34, WWN-35, WWN-37					
4.	UGEP24	3	180	GPU-28, GPU-45, PR-202, WN-467, WN-542, WN-587, WN-630	-	0.36	2.96	0.66	0.66
			195	GN-2, GN-3, GPU-67, WN-522, WN-544, WN-564, WN-584, WN-593, WN-629					
			211	GN-8, GNN-7, VL-149, WN-568, WN-602, WWN-32, WWN-34, WWN-35, WWN-37					
5.	UGEP53	3	217	GPU-28, WN-630	WN-629	0.66	2.11	0.53	0.48
			232	GN-3, GPU-67, WN-467, WN-522, WN-542, WN-587					
			247	GN-2, GN-8, GNN-7, GPU-45, PR-202, VL-149, WN-544, WN-564, WN-568, WN-584, WN-593, WN-602, WWN-32, WWN-34, WWN-35, WWN-37					
6.	UGEP67	3	72	GPU-28, VL-149, WN-467, WN-587, WN-629	PR-202, WN-522, WN-544, WN-564, WN-568 WN-584, WN-602, WN-630, WWN-34, WWN-37	0.40	4.53	0.78	0.65
			173	GN-3, GN-8, GPU-28, VL-149, WN-467, WN-542, WN-587					
			189	GN-2, GN-8, GNN-7, GPU-45, GPU-67, WN-593, WWN-32, WWN-35					
7.	UGEP93	3	161	GPU-28, WN-542, WN-587	WN-629, WWN-34	0.52	2.88	0.65	0.58
			184	GN-3, GPU-45, PR-202, WN-467, WN-522, WN-564, WN-593, WN-630					
			209	GN-2, GN-8, GNN-7, GPU-67, VL-149, WN-544, WN-568, WN-584, WN-602, WWN-32, WWN-35, WWN-37					
8.	UGEP101	3	213	GPU-28, WN-467, WN-522, WN-542, WN-587, WN-629, WN-630	-	0.48	2.73	0.63	0.63
			226	GN-2, GN-3, GN-8, GPU-45, GPU-67, PR-202, VL-149, WN-544, WN-564, WN-584, WN-593, WWN-37					
			254	GNN-7, WN-568, WN-602, WWN-32, WWN-34, WWN-35					
Average						0.48	2.91	0.62	0.61

Table 4: Correlation matrix for different parameters observed for eight markers.

	A	GR	GNR	A_e	H_e	PIC
A	1.00					
GR	0.18	1.00				
GNR	-0.18	-1.00*	1.00			
A_e	-0.33	-0.79	0.79	1.00		
H_e	-0.51	-0.64	0.64	0.96*	1.00	
PIC	0.79	-0.05	0.05	0.15	0.01	1.00

* r value significant at 5% level

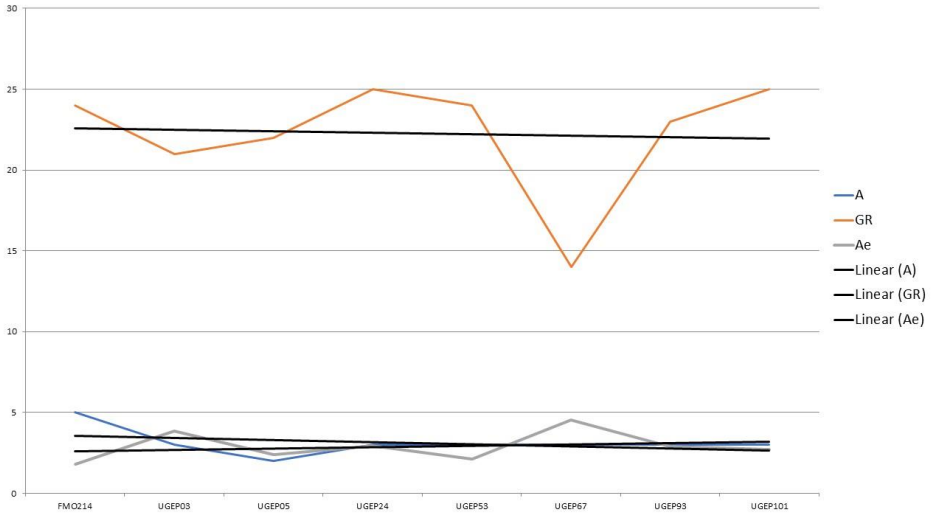


Fig. 2. Graphical representation of trends for A, A_e and GR for eight markers.

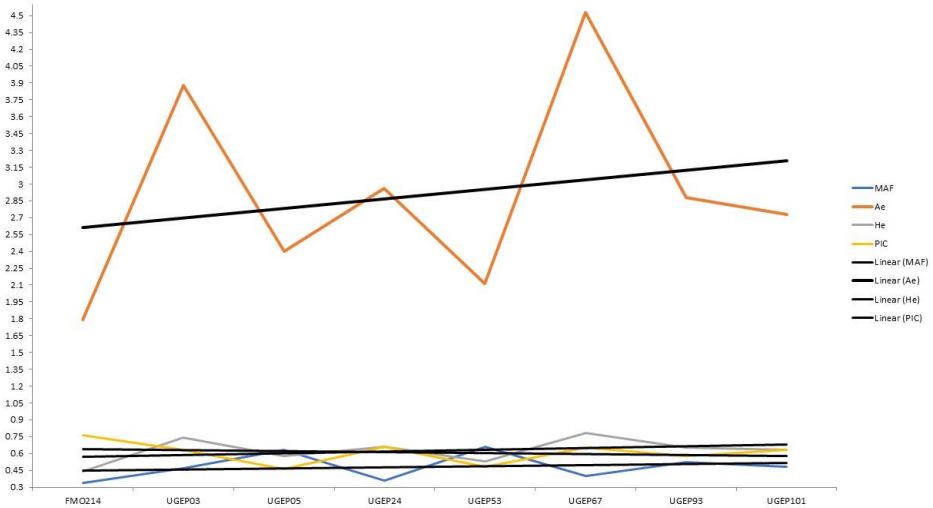
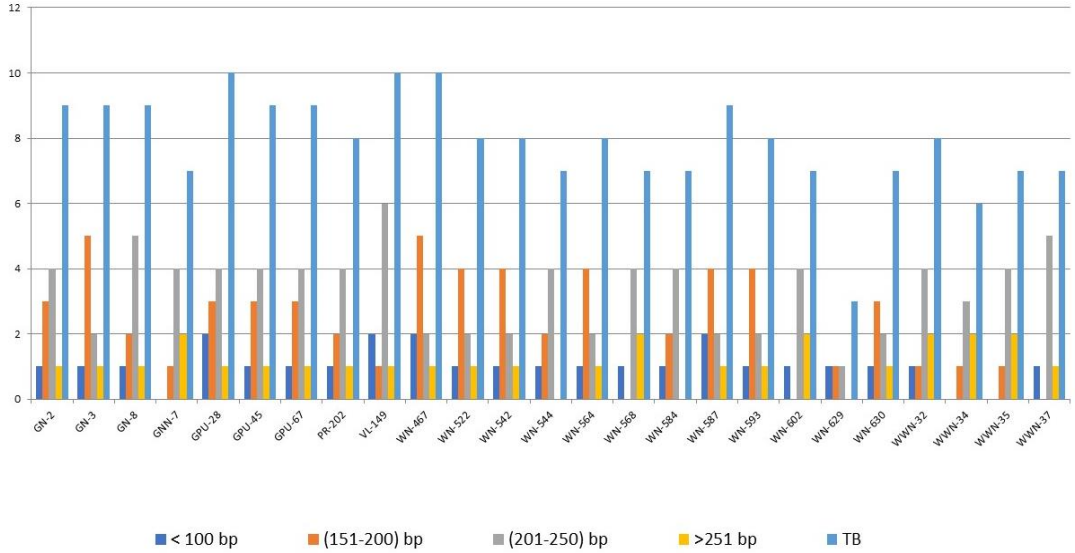


Fig. 3. Graphical representation of trends for MAF, A_e, H_e and PIC for eight markers.



TB = Total number of bands (alleles) offered by a single genotype across all markers

Fig. 4. Genotypic performance for different allelic groups.

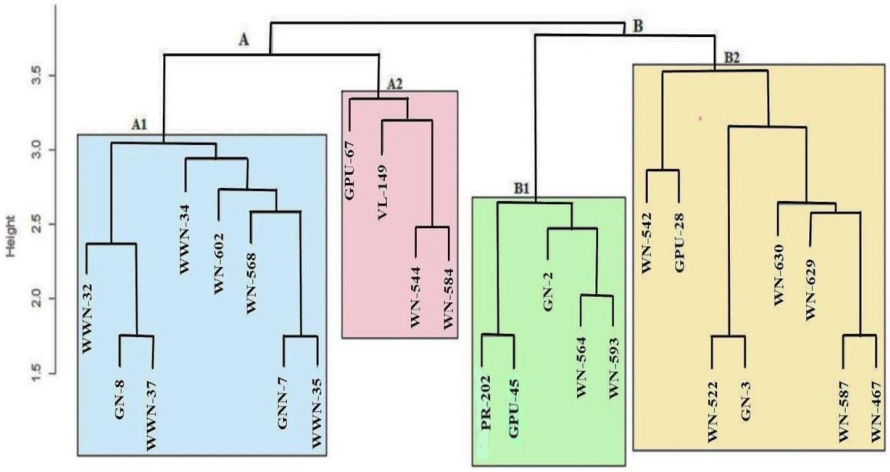


Fig. 5. Dendrogram of 25 finger millet genotypes generated from dissimilarity coefficients based on Euclidean distance.

CONCLUSIONS

More number of alleles per marker (A) will definitely lead to more number of genotypes responding per marker (GR) for one allele or the other, leading to less number of effective allele and more number of major allele frequencies and also will generate a good amount of PIC. This was actually the outcome for the marker FMO14; having highest PIC value it could be utilized best in chromosomal mapping. This outcome surely can be exploited in hybridization programme where two national checks can be crossed, or any Gujarat variety can be crossed with other belonging to two different major clusters hence ensuring considerable divergence.

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Conflict of Interest. None.

REFERENCES

Arya, L., Verma, M., Gupta, V. K. and Seetharam, A. (2013). Use of genomic and genic SSR markers for assessing genetic diversity and population structure in Indian and African finger millet (*Eleusine coracana* (L.) Gaertn.) germplasm. *Plant Systematics and Evolution*, *299*(7), 1395-1401.

Babu, B. K., Dinesh, P., Agrawal, P. K., Sood, S., Chandrashekara, C., Bhatt, J. C., and Kumar, A. (2014). Comparative genomics and association mapping approaches for blast resistant genes in finger millet using SSRs. *PloS one*, *9*(6), e99182.

Bharathi, A. (2011). Phenotypic and genotypic diversity of global finger millet (*Eleusine coracana* (L.) Gaertn.) composite collection. *Ph.D. Agriculture Thesis.* Tamil Nadu Agricultural University, Coimbatore.

De Villiers, S. M., Michael, V. N., Manyasa, E. O., Saiyiorri, A. N. and Deshpande, S. (2015). Compilation of an informative microsatellite set for genetic characterization of East African finger millet (*Eleusine coracana*). *Electronic Journal of Biotechnology*, *18*(2), 77-82.

Deshpande, S., Rangaiah, S. and Gowda, M. V. C. (2018). Assessment of molecular diversity in an elite set of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes using SSR markers. *Electronic Journal of Plant Breeding*, *9*(2), 564-576.

Dida, M. M., Wanyera, N., Dunn, M. L. H., Bennetzen, J. L. and Devos, K. M. (2008). Population structure and diversity in finger millet (*Eleusine coracana*) germplasm. *Tropical Plant Biology*, *1*(2), 131-141.

Dong, D., Fu, X., Yuan, F., Chen, P., Zhu, S., Li, B. and Zhu, D. (2014). Genetic diversity and population structure of vegetable soybean (*Glycine max* (L.) Merr.) in China as revealed by SSR markers. *Genetic Resources and Crop Evolution*, *61*(1), 173-183.

Doyle, J. J. and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, *19*(1), 11-15.

Dvořáková, Z., Čepková, P. H., Viehmannová, I., Havlíčková, L., and Janovská, D. (2016). Genetic diversity of eight millet genera assessed by using molecular and morphological markers. *Crop and Pasture Science*, *67*(2), 181-192.

Gimode, D., Odeny, D. A., De Villiers, E. P., Wanyonyi, S., Dida, M. M., Mneney, E. E. and De Villiers, S. M. (2016). Identification of SNP and SSR markers in finger millet using next generation sequencing technologies. *PLoS ONE*, *11*(7), 1-23.

Goswami, A. P., Prasad, B. and Joshi, V. C. (2015). Characterization of finger millet [*Eleusine coracana* (L.) Gaertn.] germplasm for morphological parameters under field conditions. *Journal of Applied and Natural Science*, 7(2): 836-838.

Kalyana Babu, B., Agrawal, P. K., Pandey, D., Jaiswal, J. P. and Kumar, A. (2014). Association mapping of agro-morphological characters among the global collection of finger millet genotypes using genomic SSR markers. Molecular biology reports, *41*, 5287-5297.

Karabağ, K., Balcıoğlu, M. S., Karlı, T. and Alkan, S. (2016). Determination of genetic diversity using 15 simple sequence repeats markers in long term selected japanese quail lines. *Asian-Australasian journal of animal sciences*, 29(12), 1696.

Kumar, D., Tyagi, V.; Ramesh, B. and Pal, S. (2010). Genetic diversity in finger millet (*Eleusine coracana* L.). *Crop Improvement*, *37*(1), 25-28.

Kumari, W. M. R., Pushpakumara, D. K. N. G., Weerakoon, W. M. W., Senanayake, D. M. J. B. and Upadhyaya, H. D. (2018). Morphological characterization of local and introduced finger millet (*Elusine coracana* (L.) Gaertn) germplasm in Sri Lanka. *Tropical Agricultural Research*, *29*(2), 167-183.

Laurentin, H. (2009). Data analysis for molecular characterization of plant genetic resources. *Genetic Resources and Crop Evolution*, *56*(2) 277-292.

Lee, K. J., Yoon, M. S., Shin, M. J., Lee, J. R., Cho, Y. H., Lee, H. S. and Lee, G. A. (2017). Development of SSR markers and their use in studying genetic diversity and population of finger millet (*Eleusine coracana* L. Gaertn.). *Plant Breeding and Biotechnology*, *5*(3), 183-191.

Mbinda, W. and Masaki, H. (2021). Breeding strategies and challenges in the improvement of blast disease resistance in finger millet. A current review. Frontiers in Plant Science, 11, 602882.Nei, M. (1987). *Molecular evolutionary genetics*. Columbia university press.

Nethra, N., Gowda, R., Prasad, S. R., Hittalmani, S.; Gowda, P. H. R. and Chennekeshawa, B. C. (2014). Utilization of SSR to estimate the degree of genetic relationships in finger millet (*Eleusine coracana* L. Gaertn.) genotypes and sub species. *Journal of Breeding and Genetics, 46* (1), 136-149.

Panwar, P., Nath, M., Yadav, V. K. and Kumar, A. (2010). Comparative evaluation of genetic diversity using RAPD, SSR and cytochrome P450 gene based markers with respect to calcium content in finger millet (*Eleusine coracana* L. Gaertn.). *Journal of Genetics*, *89*(2), 121-133.

Panwar, P., Saini, R. K., Sharma, N.; Yadav, D. and Kumar, A. (2010). Efficiency of RAPD, SSR and cytochrome P450 gene based markers in accessing genetic variability amongst finger millet (*Eleusine coracana*) accessions. *Molecular Biology Reports*, *37*(8), 4075-4082.

Pearson, K. *(*1895*). Notes on Regression and Inheritance in the Case of Two Parents. Proceedings of the Royal Society of London, 58,* 240-242.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. and Rafalski, A. (1996).Thecomparison of RFLP, RAPD,AFLP and SSR (microsatellite) markers for gemplasm analysis. *Molecular Breeding*, 2, 225-238.

Ramakrishnan, M., Ceasar, S. A., Duraipandiyan, V., Al-Dhabi, N. A. and Ignacimuthu, S. (2016). Assessment of genetic diversity, population structure and relationships in Indian and non-Indian genotypes of finger millet (*Eleusine coracana* (L.) Gaertn) using genomic SSR markers. *Springerplus*, *5*(1), 120.

Selvam, J. N., Muthukumar, M., Rahman, H., Senthil, N. and Raveendran, M. (2015). Development and validation of SSR markers in finger millet (*Eleusine coracana* Gaertn). *International Journal of Tropical Agriculture*, *33*(3), 2055-2066.

Singh, N., Choudhury, D. R., Singh, A. K., Kumar, S., Srinivasan, K., Tyagi, R. K., Singh, N. K. and Singh, R. (2013). Comparison of SSR and SNP markers in estimation of genetic diversity and population structure of Indian rice varieties. *PloS ONE*, *8*(12), e84136,

Sood, S., Kumar, A., Babu, B. K., Gaur, V. S., Pandey, D., Kant, L. and Pattnayak, A. (2016). Gene discovery and advances in finger millet [*Eleusine coracana* (L.) Gaertn.] genomics—an important nutri-cereal of future. Frontiers in plant science, *7*, 1634.

Sood, S., Kumar, A., Babu, B. K., Gaur, V. S., Pandey, D., Kant, L. and Pattnayak, A. (2016). Gene discovery and advances in finger millet [*Eleusine coracana* (L.) Gaertn.] genomics—An important nutri-cereal of future. *Frontiers in Plant Science*, *7*, 1634.

Suryanarayana, L., Sekhar, D. and Rao, N. V. (2014). Genetic variability and divergence studies in finger millet (*Eleusine coracana* (L.) Gaertn.). *International Journal of Current Microbiology and Applied Sciences*, *3*(4), 931-936.

Yaman, H., Tarıkahya-Hacıoğlu, B., Arslan, Y. and Subaşı, İ. (2014). Molecular characterization of the wild relatives of safflower (*Carthamus tinctorius* L.) in Turkey as revealed by ISSRs. *Genetic Resources and Crop Evolution*, *61*(3), 595-602.

Zhang, Q. P., Liu, D. C.; Liu, S., Liu, N., Wei, X., Zhang, A. M. and Liu, W. S. (2014). Genetic diversity and relationships of common apricot (*Prunus armeniaca* L.) in China based on simple sequence repeat (SSR) markers. *Genetic Resources and Crop Evolution*, *61*(2), 357-368.

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