

Research Article

SSR based genetic diversity in blast resistant and susceptible accessions of finger millet (*Eleusine coracana*.L)

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Abstract

Finger millet (*Eleusine coracana*), serves as important millets in cereals and widely cultivated mainly in arid and semiarid regions of the world. Presence of high amount of protein and calcium, it serves as a core staple food for rural populations in developing tropical countries where calcium deficiency and anemia are wide spread. In recent years, identification of polymorphisms at molecular level is being important for genetic diversity studies. Among several DNA based markers, SSR have become the marker of choice for this study. The present study was undertaken with the aim of investigating the applicability of available SSR markers for uncovered polymorphisms by using 25 primer pairs for varietal discrimination as well as for the genetic assessment in 32 finger millet accessions. Study revealed that all the SSR markers have showed polymorphism, out of which 17 were identified with a Polymorphic Information Content (PIC) value of above 0.3. The number of alleles per locus ranged from 2 to 5 with an average of 2.48 alleles per locus. Based on the similarity matrix values, the 32 genotypes were grouped into nine clusters at 0.44 Jaccard's similarity coefficient. The similarity matrix values ranged from 0.20 to 0.72. The minimum similarity was observed between Indaf-5 and GE 1330 (0.20), and maximum similarity was observed between Indaf-9 and GE 71 (0.72). Based on the SSR markers, assessment of molecular diversity could serve as a sound basis in the identification of genetically distant accessions as well as in the duplicate scoring of the morphologically close accessions. Further, the identified polymorphic markers in the current study will serve as valuable source for further breeding programmes.

Key Words

Finger millet, Blast, Accessions, Diversity, SSR markers

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.), a nutritionally important cereal crop is grown under varied agro-climatic conditions of East Africa and India. It ranks third among the millets after Sorghum and Pearl millet, and sixth among the cereals cultivated in India. India is the largest producer of finger millet with an area of 1.19 million hectares, production of 1.98 million tons per annum and productivity of 1661 kg per ha (<http://www.indiastat.com>). The native of finger millet is presumed to be highlands of central Africa, from where the crop has moved to Indian subcontinent around 3000 BC. The crop is productive in a wide range of environments and growing conditions spanning from the Himalayas in Nepal, India, and throughout the middle-elevation areas of Eastern and Southern Africa (Holt, 2000). The wide adaptability of the crop can be attributed to its C₄ photosynthetic pathway and proposed to have been an adaptation to hot, dry environments or CO₂

deficiency (Ehleringer *et al.*, 1991 and Cerling, 1999).

Growth and development of agriculture resources is mostly depending on the available genetic diversity among different crop plants and it is estimated that not even 50 per cent of the potential diversity was utilized (Brian, 1997). Therefore, identification of germplasm with distant genetic structure with their interrelationship will be very useful for future finger millet crop improvement. Such a search can be made based on geographical origin, pedigree data, morphological and molecular diversity. Evaluation of genetic diversity based on morphological characters is less precise because of the influence of extraneous factors. Hence, techniques that measure the genetic relationship without any influence of environmental factors can be made used along with required phenotypic characteristics for the breeding programme. Recent rapid advances in the field of molecular biology and its allied

sciences have made the use of molecular markers as a rapid tool for plant breeder in analyzing genetic diversity for plant improvement. Molecular marker analysis offers an efficient measure to estimate the genetic relationships on the basis of differences in genetic constitution of the genotypes Virk *et al.*(2000); Teixeira da Silva, (2005). Among the available molecular markers Simple Sequence Repeats (SSR) are most widely used to assess the genetic diversity Powell *et al.*(1996), Ma *et al.*(2001), due to their locus specificity, co-dominance, abundance, multi-allelic, high reproducibility and stability Zhang *et al.*(2007). The present study was carried out with the objective of determining the extent of genotypic difference, genetic relationship among the genotypes by using DNA markers and to select parents with divergent backgrounds to be involved in the hybridization programme.

Material and Methods

The material for present investigation was taken from global level collection of finger millet germplasm maintained at Germplasm Unit, Project Coordinating Unit (small millets), Bangalore, under the All India Coordinated Small Millets Improvement Project. The gene bank has ~7000 finger millet collections. Among them sixty four finger millet accessions were selected based on the preliminary evaluation and screening of ~ 5000 accessions which formed the initial material for the present investigation. Based on the Thirty two blast resistant and susceptible accessions of finger millet were selected based on the field evaluation during *Kharif-2011* and *Kharif-2012* at five diverse agro climatic conditions viz., Bangaluru, Ranchi, Vizianagaram, Ranichauri, and Mandy. The detailed information of the materials used for the study is given in Table 1.

Total genomic DNA was extracted from the collected leaf samples by modified CTAB (2%) method Saghai-Marouf *et al.*,(1994) and the quality with quantity of extracted DNA was checked on 0.8 % agarose with the standard lambda DNA (200 ng). PCR reactions were performed with the total reaction mixture of 10 µl consisting of 5ng template DNA, 2mM dNTPs, 25mM MgCl₂, 1X standard buffer, 10pmole of each forward and reverse primers and 3U Taq polymerase. Total reaction mixture was subjected to touch down PCR amplification with the following cycling parameters of: 94°C

for 3 min followed by 94°C for 15 s, 61°C (ramp of 1°C/cycle) for 30 s, 72°C for 30 s for 10 cycles, followed by 94°C for 15s, 58°C for 45 s, 72°C for 30 s for 40 cycles and final extension at 72°C for 10 min. The PCR products were tested for amplification on 1.2 per cent agarose stained with EtBr run in 1x TBE buffer at a constant voltage of 120 Volts for 30 - 45 min. and polymorphism was checked by using vertical PAGE (Bio-Rad genseq) unit by running 6 per cent PGE in TBE buffer. DNA ladder (NEB, USA) with a range of 100-1000 bp was used as a standard molecular marker with known weights.

PCR amplified products with clear visibility and reproducibility were scored manually on the basis of variation in fragment size. Each allele was scored as present (1) or absent (0) for each of the loci and the data analyzed for polymorphism information content (PIC) information as per Botstein *et al.*(1980).The formula, $PIC = 1 - \sum P_i^2$, where, P_i is the frequency of the i^{th} allele in the set of genotypes analysed, calculated for each locus. The genetic similarity between any two genotypes was estimated based on Jaccard's similarity coefficient (Jaccard, 1908). All the 32 genotypes were clustered with the UPGMA analysis using NTSYS-pc 2.0, Rohlf *et al.*,(2000).

Results and Discussion

Molecular techniques are best employed to assess the genetic differences existing among the genotypes. DNA markers are more useful as they are quick means of assessing genetic diversity at molecular level. In this study crop specific DNA markers including both genomic and EST derived SSR markers were used to assess the molecular diversity in blast disease resistant and susceptible accessions of finger millet. Twenty five markers were used for the diversity analysis. A total of 62 alleles were detected from 25 markers among 32 accessions (Table 2). On an average 2.48 alleles per locus were amplified among all the genotypes for SSR polymorphic markers. The marker UGEP8 (5 alleles) detected the highest number of alleles followed by UGEP12 and UGEP60 (4 alleles) (Fig-1). The PIC values ranged from 0.16 (UGEP12) to 0.75 (UGEP8) with an average of 0.33. UGEP8 showed the highest PIC values (0.75) with high number of alleles (5). Out of 25 markers, 17 markers showed the PIC values more than 0.30. With these molecular markers information, the current study has shown that these polymorphic

SSR markers could be potentially used for molecular characterization of germplasm accessions.

Based on the similarity matrix values, a dendrogram was constructed to identify genetic relationship among 32 diverse genotypes (Fig-2). Totally nine clusters were formed at 0.44 Jaccard's similarity coefficient (Table-3). Of the nine clusters, cluster IV was the largest comprising of 8 genotypes followed by cluster VII with 7 genotypes and cluster I and IX (4 genotypes), VI (3 genotypes), II and VII (2 genotypes) and III and II includes solitary genotype each as these could not be grouped with any other genotype. The similarity matrix values ranged from 0.20 to 0.72. The minimum similarity was observed between Indaf-5 and GE 1330 (0.20), and maximum similarity was observed between Indaf-9 and GE 71 (0.72).

Developmental specificity and less genome coverage of morphological traits as well as isozymes hinder their practical application of detecting polymorphism between closely related plant species. Advanced molecular marker technology has helps to reveal the real differences amongst the genotypes. DNA based molecular markers clearly allow the direct comparison of the genetic material of two individual genotypes avoiding any environmental influence due to its neutrality.

The present study addresses the utilization of 25 SSR markers to reveal genetic polymorphism and ensures identification of 32 finger millet accessions. The mean number of alleles (2.48) obtained in the present study was compared with the results of Naga *et al.*, (2012). The number of amplified products was in the range of 2 to 5 which falls within the range of 2-8 as reported by earlier workers Naga *et al.* (2012) in finger millet, Islam *et al.* (2012) in wheat (2-4 alleles) and Wang *et al.* (2012) in foxtail millet. The results showed that primer UGEP8 was able to sample five alleles at the particular marker loci whereas other primers *viz.*, UGEP12 and UGEP60 sampled four alleles each.

Genetic diversity was calculated at each locus for allelic Polymorphism Information Content (PIC). Polymorphic information content generated by the polymorphic primers ranged from 0.11 in UGEP11 to 0.75 in the UGEP8 with an average of 0.33. This was higher than that reported in

wheat (0.275 to 0.541) by Islam *et al.*, (2012) and finger millet Naga *et al.*, (2012). The SSR markers like UGEP8, UGEP12 and UGEP60 used in this study were highly informative and particularly useful in genetic diversity studies on account of their higher PIC values.

Based on Jaccard coefficients, a genetic similarity matrix was constructed using SSR marker data to assess the genetic relatedness among 32 genotypes (Table-4). This similarity matrix was used to determine the level of relatedness among the accessions of finger millet, pair-wise estimates of similarity matrix. The similarity matrix values ranged from 0.02 to 0.72. The minimum similarity was observed between Indaf-5 and GE 1330 (0.20), and maximum similarity was observed between Indaf-9 and GE 71 (0.72).

Based on similarity matrix values, five pairs each of less diverse (Indaf-9-GE 71, GE 3090-GE 496, Indaf-9-L 5, VR 708-GE 71 and VR 708-GE 1591) accessions and more diverse accessions (GE 1330-GPU 26, GE 796-GPU 48, GE 1026-GE 4449, GE 1559-GE 4440 and GE 1591-GE 4449) were identified. Further pairwise estimates of similarity value were less than 0.5 except 99 pair of accessions out of 392 pairwise estimates. This indicated that considerable genetic diversity exists in selected finger millet accessions.

Thirty two finger millet accessions were grouped into nine clusters at 0.54 Jaccard's similarity coefficient. Cluster IV was the largest comprising of eight genotypes indicating high degree of similarity between these accessions. The clustering pattern revealed that, the genotypes GPU 26, GPU 28, GPU 45 and GPU 66 were almost identical with high similarity coefficient and is grouped within the cluster IV at molecular level and susceptible genotype KM 252 PR 202 and K 7 are grouped into similar cluster. These results suggest that even the two different genotypes derived from different parentage may share some similar genomic regions as common.

With this study it has been observed that a considerable diversity exist among the selected finger millet germplasm accessions. The results concluded that the co-dominant SSR markers are much more useful in revealing the unique allelic

variation in finger millet for understanding their evolutionary significance. Analysis of genetic diversity in germplasm collection will help future breeding for meeting human demands by guiding in improving yield potential through selection of diverse parents in crop improvements.

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Table 1. List of 32 genotypes of finger millet taken for diversity analysis with their characteristic features

Sl. No.	Entry	Pedigree	Origin	Disease reaction
1	GE 496	IE 316	India (MP)	Resistant
2	GE 3090	SAD8511403	ICRISAT	Resistant
3	GE 4440	-	Orissa	Resistant
4	GE 4449	-	Orissa	Resistant
5	GPU 26	Indaf 5 ×(derived line of indaf 9 X IE 10121	PC Unit, Bangalore	Resistant
6	GPU 28	Indaf 5 × (Derived line of Indaf 9 X IE 1012)	PC Unit, Bangalore	Resistant
7	GPU 45	GPU 26 ×L5	PC Unit, Bangalore	Resistant
8	GPU 48	-	PC Unit, Bangalore	Resistant
9	GPU 66	PR-202 × GPU 28	PC Unit, Bangalore	Resistant
10	GE 132	IE329)	India (MP	Resistant
11	GE 1787	IE 927		Resistant
12	GE 1026	HR 23-8-9	Karnataka	Resistant
13	GE 1330	P655	Africa	Resistant
14	GE 1356	P 82	Africa	Resistant
15	GE 1382	P-662	Africa (Zambia)	Resistant
16	GE 1402	Orissa	Dibya sinha	Resistant
17	GE 1559	IE990	-	Resistant
18	GE 1591	-	-	Resistant
19	GE 71	IE 927	-	Resistant
20	GE 569	IE339	India (TN)	Resistant
21	GE 669	(IE1012)	Africa (Zambia)	Resistant
22	VR 708	Pure Line Selection	Vizianagaram	Susceptible
23	L 5	Malavi × Indaf 9	ARS, Nagenahalli	Susceptible
24	Indaf 9	K 1 × IE 980 R	V.C. farm, Mandya	Susceptible
25	Indaf 5	Cavery × IE 927	V.C. farm, Mandya	Susceptible
26	Purna	Co 1 × Aruna	Dept of Agri, Karnataka	Susceptible
27	PES 110	Pure line selection	Pantanagar	Susceptible
28	PR 202	Pure line selection	Peddapuram	Susceptible
29	KM 252	-	-	Susceptible
30	K 7	Cross derivative of Co 8 × K 2	TNAU, Coimbatore	Susceptible
31	VL 149	VL_204 ×IE 882	Almora	Susceptible
32	GE 796	IE 460	-	Susceptible



Table 2. Primer name, Sequences, Annealing Temperature, Product Size (bp), Allele richness and polymorphic information content (PIC) of the SSR markers in selected finger millet accessions

Sl. No.	Primer Name	Primer Sequence	Annealing temperature	Product Size (bp)	Alleles/ locus	PIC
1	UGEP1F	TTCAGTGGTGACGGAAGTTCT	58.75	233	2	0.30
	UGEP1R	GGCTCCATGAAGAGCTTGAC				
2	UGEP5F	TGTACACAACACCACACTGATG	58.20	215	2	0.32
	UGEP5R	TTGTTTGGACGTTGGATGTG				
3	UGEP6F	AGCTGCAGTTTCAGTGGATTC	58.25	229	2	0.36
	UGEP6R	TCAACAAGGTGAAGCAGAGC				
4	UGEP8F	ATTTCCGCCATCACTCCAC	58.65	297	5	0.75
	UGEP8R	AGACGCAAAATGGGTAATGTC				
5	UGEP10F	AAACGCGATGAATTTTAAGCTC	58.55	400	3	0.40
	UGEP10R	CTATGTCGTGCCCATGTCCG				
6	UGEP11F	CCTCGAGTGGGGATCCAG	59.40	153	2	0.11
	UGEP11R	AAGACGCTGGTGGAAATAGC				
7	UGEP12F	ATCCCCACCTACGAGATGC	58.95	230	4	0.65
	UGEP12R	TCAAAGTGATGCGTCAGGTC				
8	UGEP15F	AAGGCAATCTCGAATGCAAC	59.05	180	3	0.46
	UGEP15R	AAGCCATGGATCCTTCCTTC				
9	UGEP18F	TTGCATGTGTTGCTTTTTCG	58.65	318	2	0.23
	UGEP18R	TGTTCTTGATTGCAAACCTGATG				
10	UGEP21F	CAATTGATGTCATTGGGACAAC	59.55	225	2	0.16
	UGEP21R	GTATCCACCTGCATGCCAAC				
11	UGEP24F	GCCTTTTGATTGTTCAACTCG	58.70	183	3	0.42
	UGEP24R	CGTGATCCCTCTCCTCTCTG				
12	UGEP31F	ATGTTGATAGCCGAAATGG	58.60	241	2	0.30
	UGEP31R	CCGTGAGCCTCGAGTTTTAG				
13	UGEP52F	TCATGCTAGCTTCAACACAACC	59.00	215	2	0.36
	UGEP52R	TGCTGGGTGAAACCCTAGAC				
14	UGEP53F	TGCCACAACCTGTCAACAAAAG	58.95	226	2	0.37
	UGEP53R	CCTCGATGGCCATTATCAAG				
15	UGEP60F	AGCTCTGCTTGGTGGAGAAG	58.75	240	4	0.44
	UGEP60R	TTTTCTACTGGTGGGCGAAG				
16	UGEP68F	CGGTGAGCATATAACGAATGG	59.25	232	2	0.19
	UGEP68R	TCATTGATGAATCCGACGTG				
17	UGEP77F	TTCGCGCGAAATATAGGC	58.55	245	3	0.38
	UGEP77R	CTCGTAAGCACCCACCTTTC				
18	UGEP81F	AAGGGCCATACCAACTCC	59.80	192	2	0.37
	UGEP81R	CACTCGAGAACCGACCTTTC				
19	UGEP102F	ATGCAGCCTTTGTCATCTCC	59.00	184	2	0.16
	UGEP102R	GATGCCTTCCTTCCCTTCTC				
20	UGEP104F	TCAGCACCACTGAATAGG	57.30	189	2	0.16
	UGEP104R	AATAGGGAGGGCGAAGACTC				
21	UGEP106F	AATTCCATTCTCTGCATCG	59.20	175	2	0.26
	UGEP106R	TGCTGTGCTCCTCTGTTGAC				
22	UGEP110F	AAATTCGCATCCTTGCTGAC	59.10	192	2	0.33
	UGEP110R	TGACAAGAGCACACCGACTC				
23	FMMA541a	TTGCCAATTTTCGATCTTACT	55.00	232	3	0.33
		GCAAAGGATACTCTCCCTCT				
24	FMMA5005	TCCCCTACATCCAGTTCTCG	50.00	184	2	0.28
		GGGTCCCTTCCCCTTAGAGT				
25	FMMA541c	GAGGGAGAGTATCCTTTGCT	54.00	131	2	0.32
		AGTAAGATCGAAACACGGAA				
				Total	62	
				Average	2.48	0.33



Table 3. SSR marker assay based clustering pattern of selected accessions of finger millet

Clusters	No. of Entries	Genotypes
I	4	GE 496, GE 3090, GE 4440 and GE 569
II	2	GE 4449 and GPU 48
III	1	PES 110
IV	8	GPU 26, GPU 28, GPU 45, GPU 66 GE 1356, GE 132, GE 1787 and Poorna
V	1	GE 1026
VI	3	PR 202, KM 252 and K7
VII	2	GE 1402 and GE 1559
VII	7	GE 1591, VR 708, GE 71, Indaf 9, L5, Indaf 5 and VL 149
IX	4	GE 1330, GE 1382, GE 669 and GE 796

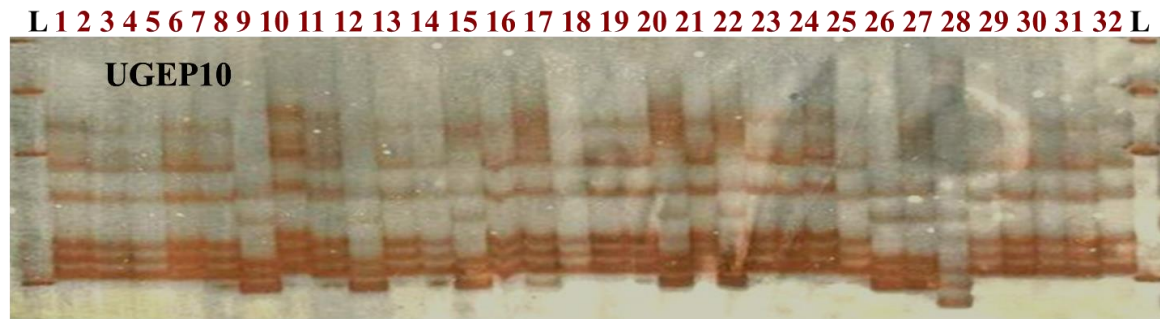


Fig. 1. Molecular profiling of Marker UGEP10 in finger millet accessions

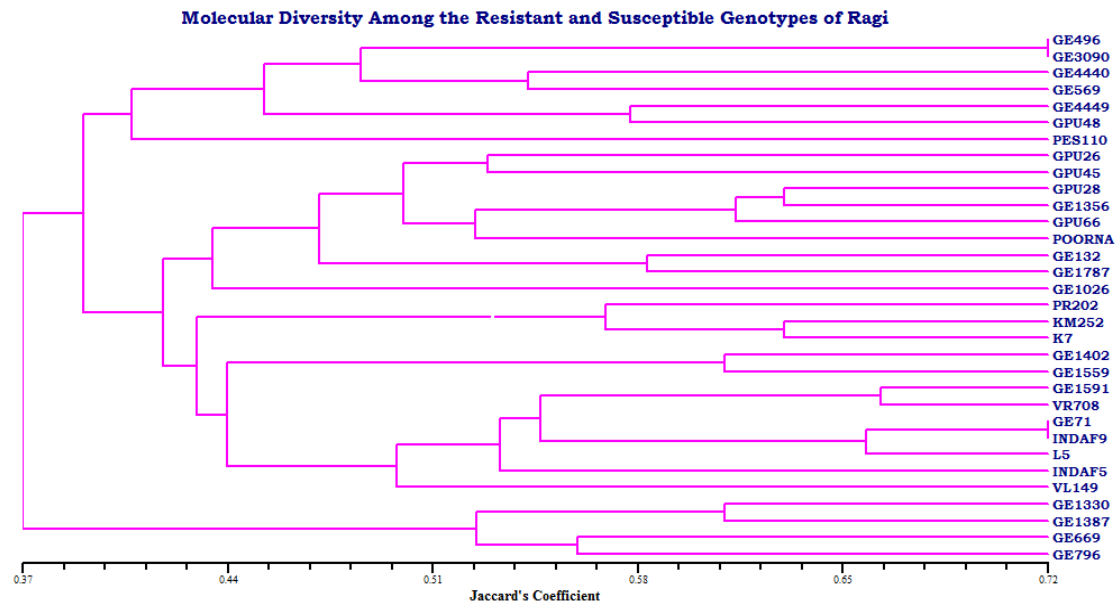


Fig. 2. Genetic Clustering of selected 32 diverse finger millet genotypes with the UPGMA analysis using the NTSYS-PCv2.10t (Rolf, 2010).