RESEARCH ARTICLE

SABRAO Journal
of Breeding and Genetics
46 (1) 136-149, 2014

UTILIZATION OF SSRS TO ESTIMATE THE DEGREE OF GENETIC RELATIONSHIPS IN FINGER MILLET (*Eleusine coracana* L. Gaertn.) GENOTYPES AND SUBSPECIES

N. NETHRA[1]*, RAME GOWDA[1], S. RAJENDRA PRASAD[2], S. HITTALMANI[3], P.H. RAMANJINI GOWDA[4] and B.C. CHENNAKESHAVA[1]

[1]AICRP on Seed Technology, NSP, University of Agricultural Sciences, GKVK, Bangalore-560065, India
[2]Directorate of Seed Research, MAU, UP, India
[3]Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK, Bangalore-560065, India
[4]Department of Biotechnology, University of Agricultural Sciences, GKVK, Bangalore-560065, India
*Corresponding author's email: nethraharsha@gmail.com

SUMMARY

Genotypic analysis of 24 cultivated and wild species of finger millet was carried out using 35 simple sequence repeat (SSR) markers. Genetic relationship among diverse genotypes was established using morphological and molecular variations. An average of 3.11 alleles per SSR marker and 34% polymorphic information content were detected for the 24 genotypes. The important quantitative traits contributing to diversity was highest for number of grains/spikelet followed by plant height. The lowest genetic diversity was observed within the cultivated finger millet, whereas the wild species showed the highest genetic diversity and also contains several desirable alleles as the wild species showed higher values for important yield related traits. Therefore, it is recommended that interspecific hybridization and introgression should be used in breeding. SSR markers were more efficient in detection of polymorphism as they are multi-allelic.

Keywords: Finger millet, SSR, genetic relationship, polymorphism, morphology

Manuscript received: June 12, 2013; Decision on manuscript: November 4, 2013; Manuscript accepted: April 12, 2014.

Communicating Editor: Bertrand Collard

INTRODUCTION

Finger millet, *Eleusine coracana* L. Gaertn., is a tetraploid crop (2*n*=4*x*=36; genome constitution AABB) belonging to the grass family *Poaceae*, subfamily Chloridoideae commonly called as Ragi in India. It ranks fourth after pearl millet (*Pannicum glaucum*), foxtail millet (*Setaria italica*) and proso millet (*Panicum miliaceum*) with an approximately 8% of the area and 11% of the production in the world. About 4.5 million tons of grains are produced annually on 5 million hectares of land throughout the world (FAO, 2011). India alone produces 40-45% of the total world production with 2.04 million tones grains on 1.41 million hectares of land with a productivity of 1,477 kg per hectare (Anonymous, 2009).

Finger millet is an important dry land crop and is grown for its dual use as a source of 'food grain' and 'stover'. The grain contains 70-76% of carbohydrates, 7-14% crude protein and particularly rich in methionine, iron and calcium (Barbeau and Hilu, 1993; Vadiuoo, 1998). It has

an inherent capacity to tolerate several biotic and abiotic stresses including water stress. Hence, it is one of the important crops for dryland agriculture and nutritional requirement of poor and marginal farmers of the country. Therefore, continuous efforts are required to improve the productivity by improving the genetic potential of the varieties. In this direction, the modern tools and techniques applied in major crops to improve the productivity and adaptability are to be applied in orphan crops like finger millet.

India is having a rich source of germplasm which has been conserved (Ramakrishna *et al.,* 1996). However, only a small fraction of the genetic diversity is utilized in crop improvement program of the country. Although, most of the germplasm has been characterized for morphological traits, the information on utilization of DNA markers to assess the diversity is very limited. Many breeding programs for major crops use molecular markers (Kottearachchi, 2013) largely due to rapid growth in genomic research. However the molecular studies in finger millet are scanty and most of the studies are based on RAPD markers which are not very useful (Fakrudin *et al*., 2004).

In crops, microsatellite markers have been very useful to study genetic relationships (Gupta and Varshney, 2000), tagging genes or quantitative trait loci (QTLs) for important traits (Collard *et al.,* 2005) and for MAS because of their high polymorphism level and codominant inheritance. The plant SSRs are reported to exhibit high levels of polymorphism with as many as 37 alleles at individual loci in barley (*Hordeum vulgare* L.*)* (Saghai-Maroof *et al.,* 1994) and 26 alleles in soybean (Rongwen *et al.,* 1995*)*. However, SSR marker resources available in finger millet are very limited. The development of robust SSR markers has not been attempted in finger millet. Recently a few studies were conducted to develop SSR in finger millet (Dida *et al.,* 2007). SSR markers can be effectively used for diversity studies (Dida *et al.,* 2008) and phylogenetic analysis.

The paper describes the analysis of a diverse collection of finger millet genotypes including cultivated and related species for polymorphic information content of SSR markers. In addition, genetic relationship among diverse genotypes was established using morphological and molecular variation.

MATERIALS AND METHODS

Plant material

Highly productive 22 cultivated varieties of *Eleusine coracana* subsp. *coracana*, 1 *E. coracanasub sp.africana* and 1 *E. indica* accessions were obtained from the project co-ordination cell, All India coordinated small millets improvement project (AICSMIP), UAS, GKVK, Bangalore for the present study (Table 1).

Phenotypic evaluation of genotypes

The selected accessions were grown in a randomized complete block design with 3 replications during 2007 kharif seasons at GKVK, Bangalore. Each entry was grown in a single row of 5 m length per replication. Standard agronomic practices were followed. Ten plants per entry were randomly selected to record observations on the following 22 characters: plant height, number of productive tillers, leaf number, flag leaf blade length and width, flag leaf sheath length and width, peduncle length, finger number, finger length, days to 50% flowering, days to maturity, 1000-seed weight, number of grains/spikelet, growth habit, plant pigmentation, ear shape, finger branching, gaps on finger, seed color, seed shape and seed surface by following IBPGR descriptor (Harinarayana and Seetharam, 1985).

Genotypic characterization

DNA Extraction

For each genotype, 15 seeds were sown in a pot and grown in the green house in disease free conditions until 3-4 leaves developed. An equal quantity of leaf material from 10 plants was harvested, bulked, lyophilized, and finely ground with pestle and mortar. Genomic DNA was extracted using modified CTAB procedure (Saghai-Maroof *et al.,* 1984).

Table 1. List of finger millet genotypes and wild accessions.

No.	Genotypes	Pedigree	Geographical origin
1	Indaf-5	Cauvery X IE 927	Karnataka
2	Indaf-7	Annapurna X IE 927	Karnataka
3	Indaf-8	Hullubele X IE 929	Karnataka
4	Indaf-9	KI X IE 98R	Karnataka
5	GPU-26	Indaf-5 X Indaf-9	Karnataka
6	GPU-28	Indaf-5 X Indaf-9	Karnataka
7	GPU-45	GPU-26 X L-5	Karnataka
8	GPU-48	GPU-26 X L-5	Karnataka
9	HR-911	UAS 1 X IE 927	Karnataka
10	HR-374	EE 4842 X IE 927	Karnataka
11	L-5	Malawi X Indaf-9	Karnataka
12	MR-1	Hamsa X IE 927	Karnataka
13	MR-6	African white X ROH-2	Karnataka
14	ML-181	IE 1012 X I-5	Karnataka
15	ML-322	IE 1012 X I-5	Karnataka
16	PR-202	Selection from local cultivar Mettachodi	Andhra Pradesh
17	PES-110	Selection from local cultivar Pantnagar	Uttar Pradesh
18	PES-400	Selection from local germplasm	Uttar Pradesh
19	RAU-8	BR 407 X Ranchi	Bihar
20	VR-708	Pure line selection from VMEC 36	Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Orissa and Karnataka
21	VL-149	VL 204 X IE 882	Uttar Pradesh
22	VL-305	SDFM 69 X VL-231	Uttar Pradesh
23	*E. africana*	Africa	-
24	*E. indica*	India	-

SSR marker analysis

Microsatellite marker (SSR) analysis was conducted using 35 primer pairs (Table 2) developed by Dida *et al.* (2007) in finger millet. The PCR reactions were carried out in a final volume of 20 µl containing - PCR buffer 1x, 1.25 mM $MgCl_2$, 100 ng template DNA, 500 nM each of the forward and reverse primer, 200 µM dNTPs and 0.8 U of Taq DNA polymerase. Amplifications were conducted using a touchdown program in an MJ PTC-200 thermal cycler. The initial denaturation at 94 ^{0}C for 3 min followed by 10 cycles of denaturation at 94 ^{0}C for 30 s, touchdown annealing starting at 62 ^{0}C for 30 s and decreasing 0.7 ^{0}C/cycle, and extension at 72 ^{0}C for 1 min, this was followed by a further 35 cycles at an annealing temperature of 55 ^{0}C. The program finished with a final extension at 72 ^{0}C for 4 min.

Gel electrophoresis

The amplified PCR products were resolved by running on 3.5% agarose and 6% PAGE gels separately, electrophoresis in TBE buffer. DNA was visualized by staining with ethidium bromide and viewing using gel documentation system (Gel Doc 2000, Bio Rad).

Table 2. List of SSR primers and their sequences, nucleotide repeat length and amplified product size analyzed in the study.

Primer	Primer sequence 5^1 to 3^1	SSR motif	Molecular size (bp)
UGEP1F	TTCAGTGGTGACGGAAGTTCT	$(TC)_{11}$	233
UGEP1R	GGCTCCATGAAGAGCTTGAC		
UGEP3F	CCACGAGGCCATACTGAATAG	$(CA)_7N_{12}(GA)_{15}$	206
UGEP3R	GATGGCCACTAGGGATGTTG		
UGEP5F	TGTACACAACACCACACTGATG	$(TC)_{12}AC(TC)_4$	215
UGEP5R	TTGTTTGGACGTTGGATGTG		
UGEP6F	AGCTGCAGTTTCAGTGGATTC	$(GA)_3TA(GA)_9$	229
UGEP6R	TCAACAAGGTGAAGCAGAGC		
UGEP8F	ATTTCCGCCATCACTCCAC	$(GA)_{13}$	297
UGEP8R	AGACGCAAATGGGTAAATGTC		
UGEP10F	AAACGCGATGAATTTTAAGCTC	$(GA)_{19}$	400
UGEP10R	CTATGTCGTGTCCCATGTCG		
UGEP11F	CCTCGAGTGGGGATCCAG	$(CT)_{12}$	153
UGEP11R	AAGACGCTGGTGGAAATAGC		
UGEP12F	ATCCCCACCTACGAGATGC	$(CT)_{22}$	230
UGEP12R	TCAAAGTGATGCGTCAGGTC		
UGEP15F	AAGGCAATCTCGAATGCAAC	$(CT)_{22}$	180
UGEP15R	AAGCCATGGATCCTTCCTTC		
UGEP18F	TTGCATGTGTTGCTTTTTGC	$(CT)_{12}$	318
UGEP18R	TGTTCTTGATTGCAAACTGATG		
UGEP21F	CAATTGATGTCATTGGGACAAC	$(GA)_{16}$	225
UGEP21R	GTATCCACCTGCATGCCAAC		
UGEP24F	GCCTTTTGATTGTTCAACTCG	$(GA)_{26}$	183
UGEP24R	CGTGATCCCTCTCCTCTCTG		
UGEP26F	ATGGGGTTAGGGTTCGAGTC	$(CGG)_7$	227
UGEP26R	TGTCCCTCACTCGTCTCCTC		
UGEP31F	ATGTTGATAGCCGGAAATGG	$(GA)_{12}$	241
UGEP31R	CCGTGAGCCTCGAGTTTTAG		
UGEP52F	TCATGCTAGCTTCAACACAACC	$(GA)_{16}$	215
UGEP52R	TGCTGGGTGAAACCCTAGAC		
UGEP53F	TGCCACAACTGTCAACAAAAG	$(AG)_{26}$	226
UGEP53R	CCTCGATGGCCATTATCAAG		
UGEP56F	CTCCGATACAGGCGTAAAGG	$(GT)_{12}$	162
UGEP56R	ACCATAATAGGGCCGCTTG		
UGEP60F	AGCTCTGCTTGGTGGAGAAG	$(GA)_{37}$	240
UGEP60R	TTTTCTACTGGTGGGCGAAG		
UGEP65F	AGTGCTAGCTTCCCATCAGC	$(CT)_{19}$	226
UGEP65R	ACCGAAACCCTTGTCAGTTC		
UGEP68F	CGGTCAGCATATAACGAATGG	$(CT)_{14}$	232
UGEP68R	TCATTGATGAATCCGACGTG		

UGEP76F	GCACGTACGGATTCACATTG	$(CAG)_7$	168
UGEP76R	GGTACGGAGACATCGACACC		
UGEP77F	TTCGCGCGAAATATAGGC	$(CT)_{19}$	245
UGEP77R	CTCGTAAGCACCCACCTTTC		
UGEP78F	AAGCAATCAACAAAGCCTTTTC	$(GA)_{14}$	244
UGEP78R	TACAACGTCCAGGCAACAAG		
UGEP81F	AAGGGCCATACCAACACTCC	$(GT)_{12}$	192
UGEP81R	CACTCGAGAACCGACCTTTG		
UGEP90F	GGCCTTTGCAGTCATGTGAG	$(CT)_{11}/(CT)_8$	232
UGEP90R	CGACTCCAGGTGTTGTTGG		
UGEP102F	ATGCAGCCTTTGTCATCTCC	$(TG)_{17}$	184
UGEP102R	GATGCCTTCCTTCCCTTCTC		
UGEP104F	TCAGCACCACCTGAATAGG	$(CT)_{11}$	189
UGEP104R	AATAGGGAGGGCGAAGACTC		
UGEP106F	AATTCCATTCTCTCGCATCG	$(AC)_{12}$	175
UGEP106R	TGCTGTGCTCCTCTGTTGAC		
UGEP107F	TCATGCTCCATGAAGAGTGTG	$(GA)_{15}$	224
UGEP107R	TGTCAAAAACCGGATCCAAG		
UGEP108F	GTTGGCTGCTCTGCTTATCC	$(CTG)_6(CAG)_2$	150
UGEP108R	TATCTGCTTGTGCAGCTTCG		
UGEP110F	AAATTCGCATCCTTGCTGAC	$(CT)_{12}$	192
UGEP110R	TGACAAGAGCACACCGACTC		

The PCR products in PAGE were visualized by silver staining according to the protocol described by Bassam *et al*. (1991). For SSR genotyping, the DNA finger-prints were scored manually. Each allele was scored as present (1) or absent (0) for each of the SSR loci.

Statistical Analysis

Both morphological and molecular marker data were recorded in a spreadsheet arranging genotypes and traits/-markers in columns and rows, respectively.

Morphological data analysis

The field data on morphological traits was analyzed by ANOVA with 24 treatments and 2 replications to compare the genotypes (Panse and Sukhatme, 1961).

Morphological data were standardized as:

$$X'_{ki} = (X_{ki} - X_{\min})/(X_{\max} - X_{\min})$$

and phenotypic distances were calculated as the Manhattan coefficient

$$D_{ij} = 1/n \sum_{k=1}^{n} |X'_{ki} - X'_{kj}|,$$

where X_{ki} and X_{kj} are the observed values of the two lines i and j with respect to the k^{th} variable and n is the number of variables considered. The distances calculated according to this procedure are suitable when both metric and qualitative traits are considered. From phenotypic distances, phenotypic similarities were obtained as $1 - D_{ij}$ and used for cluster analysis. All calculations were performed using the subprograms STAND, SIMINT, and TRANSF of the statistical package NTSYS-pc, version 2.0 (Rohlf, 2000).

Polymorphic Information content (PIC) for SSR markers

The PIC values measure the informativeness of a given DNA marker, and these were calculated as follows:

$$PIC = 1 - \sum_{i=1}^{k} Pi^2$$

where k is the total number of alleles detected for a given marker locus and Pi is the frequency of the i^{th} allele in the set of genotypes investigated.

Genetic Similarity

Similarity was calculated using the simple matching (SM) coefficient (Sneath and Sokal, 1973). Similarity matrices were obtained using the sub-program SIMQUAL (NTSYS-pc, version 2.0 (Rohlf, 2000). Finally, from the 3 types of similarity matrices, clusters were built using the unweighted pair group method with arithmetic average (UPGMA) procedure in the SAHN subprogram, considering all the markers showing polymorphism among the 22 cultivars and 2 wild species.

RESULTS AND DISCUSSION

Thirty five SSR primers were used to characterize and evaluate 24 genotypes of finger millet of which 2 were wild species. All the primers produced polymerase chain reaction amplicons within all the genotypes. As expected, more polymorphisms were observed using PAGE and could able to differentiate microsatellite loci with a few base pair difference. Therefore, the data obtained from PAGE was used for further analysis. The total number of bands produced was 109 with a mean of 3.1 per primer. The number of alleles revealed by each primer is presented in Table 3 and Figure 1. The number of alleles produced by each SSR varied from 1 to 6. The majority of the primers produced more than 2 alleles. The markers used in the present study consisted of both genic and genomic SSRs (Dida, 2007). The genomic SSRs are more informative for fingerprinting and estimation of genetic diversity because of their dispersion throughout the genome (Kuleung *et al.,* 2004). The high number of alleles per locus among the tested genotypes suggests a broad genetic base used in this study.

The largest number of alleles was observed in the wild species *E. indica* followed *by E. africana* species. This may be due to the presence of unique alleles present in wild genotypes, which have been lost during the cultivation and adaptation to favorable conditions (Sundaram *et al*., 2007). These results like many studies in other crops demonstrate the utility of wild germplasm for broadening the genetic base of finger millet.

Of the 35 primers tested, 4 primers produced monomorphic bands in all the tested cultivars including wild species. Sixteen primers produced monomorphic bands among cultivated genotypes but produced polymorphic bands in wild species. The remaining primers produced polymorphic bands in all the tested genotypes. The results from the limited genotypic analysis using 35 SSR markers indicate that the wild species are genetically distinct and diverse from the cultivated species. The domestication has reduced the genetic variation among cultivated genotypes.

The higher genetic variation in wild finger millet species could be due to considerable amount of natural out crossing observed in the wild species (de Wet *et al.,* 1984). The cultivated genotypes are derived from continuous inbreeding and selection from a limited number of founder populations, which would have lowered genetic diversity. The 22 cultivated genotypes are high yielding varieties from different parts of India particularly Karnataka hence, have lower genetic variation (Table 1). The PIC values of SSR primers ranged from 0% (UGEP 5, 26, 55, and 109) to 85% (UGEP 25) followed by 70% (UGEP 15).

The similarity indices and consensus in formativeness were developed on the basis of scorable banding patterns of 24 genotypes using 35 primers and are presented in Figure 2. The similarity indices divided the 24 genotypes into 2 main clusters with a similarity coefficient of 0.29.

Table 3. Distribution of number of alleles and PIC per cent in different primers.

SSR Primers	No. of Alleles	PIC (%)
UGEP 1	2	16
UGEP 3	4	56
UGEP 5	1	0
UGEP 6	6	57
UGEP 8	3	19
UGEP 10	3	53
UGEP 11	3	8
UGEP 12	4	56
UGEP 15	5	70
UGEP 18	4	16
UGEP 19	2	47
UGEP 21	4	40
UGEP 24	5	61
UGEP 25	5	85
UGEP 26	1	0
UGEP 31	2	22
UGEP 55	1	0
UGEP 53	3	51
UGEP 56	5	36
UGEP 60	3	66
UGEP 65	4	58
UGEP 68	3	48
UGEP 73	2	8
UGEP 76	2	4
UGEP 77	3	16
UGEP 78	4	24
UGEP 81	4	53
UGEP 90	2	8
UGEP102	3	23
UGEP104	2	15
UGEP106	5	66
UGEP107	2	4
UGEP108	3	16
UGEP109	1	0
UGEP110	3	16
Mean	3.11	34

PIC: Polymorphic Information Content

One cluster included the 2 wild species *E. africana* and *E. indica* and the other cluster comprised of all the cultivated genotypes. The similarity between these 2 clusters was lowest with a similarity coefficient of 0.29.

Although, the 2 wild species shown to be in the same cluster the similarity index between these 2 species was low (0.45). The second cluster consisted of cultivated species was also further divided in to several sub groups using SSR markers. The 2 genotypes MR-1 & MR-6 originated from Karnataka from the same station, and were most closely related with a similarity index of 0.96. The SSR markers show a much higher level of polymorphism and are more informative. The wild species with most distinct DNA profiles are likely to contain the greatest number of novel genes (Dida *et al.,* 2008). Similarity co-efficient among cultivated genotypes obtained from different parts of the

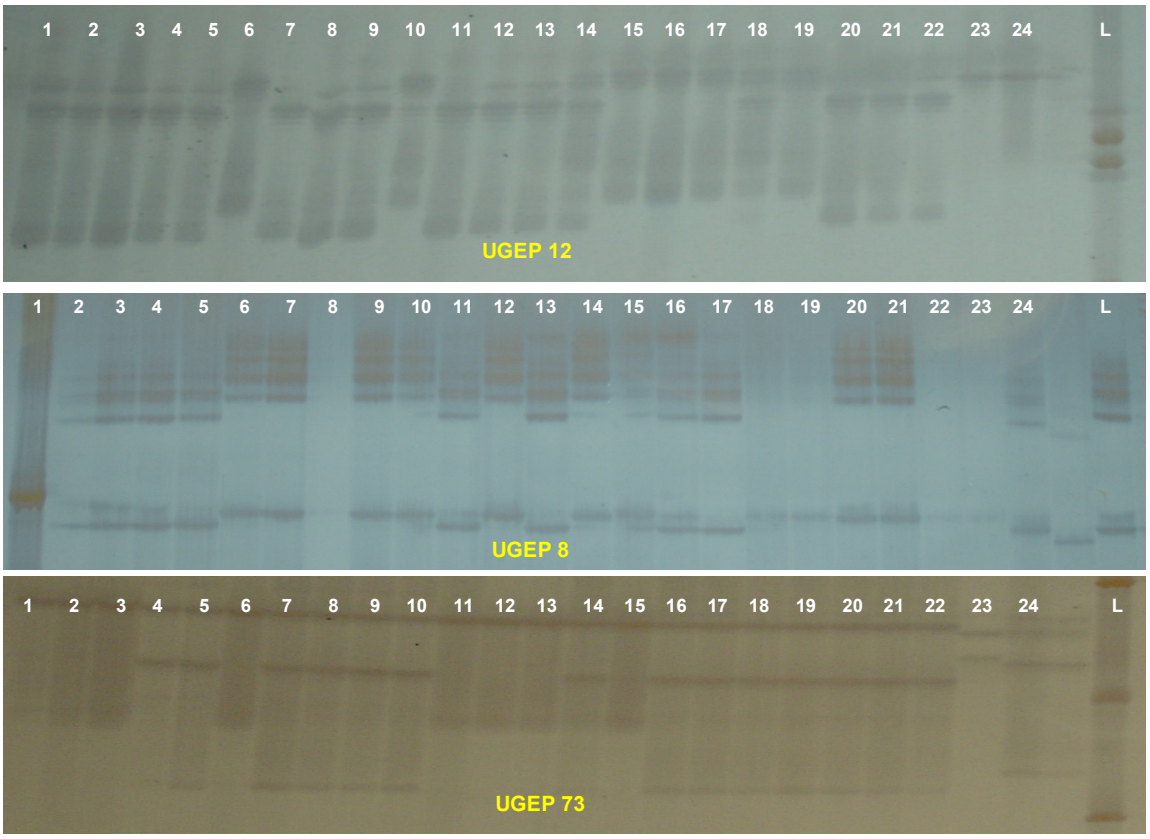
country was moderate (0.75). The diversity determined by SSR markers is an indicator of usefulness of microsatellite markers in identifying the unique genotypes for favorable recombination in recombination breeding.

The diversity analysis of 22 characters based on both qualitative and quantitative phenotypic characters was carried out and the dendrogram of 24 genotypes is presented in Figure 3. The genotypes were classified broadly in 2 groups. The first cluster included only 2 wild species *E. africana sub sp. africana* and *E. indica*. The wild species were distinct from the cultivated genotypes in morphology. The domestication and adaptation of the cultivated genotypes drifted the genotypes by reducing the number of alleles. The difference in the mean values (Table 4) of the quantitative traits for cultivated and wild species contributed for genetic divergence in this study.

Among the quantitative traits diversity was highest in number of grains per spikelet (31.62) followed by plant height (15.02), 1000 seed weight (13.44), finger length (10.28), peduncle length (8.70), days to maturity (7.51) 50% flowering (6.72), number of productive tillers (5.00) and finger numbers (5.00). The difference between cultivated and wild species was also significant for the above important traits (Table 4). For example; the number of grains per spikelet was 4.56 in cultivated species as compared to 8.25 of wild species. Similarly, for plant height 97.95 in cultivated species compared to 149.25 in wild species. It is interesting to note that the wild species showed higher values for all the productivity traits except 1000 seed weight. Hence, the wild species are not only genetically diverse from the cultivated types but also contain several desirable alleles. Therefore, it is recommended to practice interspecific hybridization and introgression breeding. The inter-specific hybridization in finger millet is expected through wide variation as the wild species are genetically distinct as indicated. The reports on introgression breeding in finger millet breeding are limited.

The second cluster comprising of cultivated genotypes were further subdivided in to 2 subgroups. The subgroup-1 comprised of only 2 genotypes L-5 and HR 374. The second group had a large number of varieties which was further divided into many subgroups. However, the grouping of genotypes did not follow any definite pattern. The knowledge about genetic relationships of genotypes provides useful information to address breeding strategies and germplasm resource utilization.

In the past, a variety of molecular markers such as RAPDs and AFLPs have been used for estimating the genetic diversity in finger millet (Fakrudin *et al.,* 2004; Das *et al.,* 2006; Preety *et al.,* 2010). Recently SSR markers, EST markers, resistant gene analogue markers were also used to evaluate genetic diversity in finger millet (Dida *et al.,* 2007 and 2008 and Srinivasachary *et al*., 2007). The use of a specific type of molecular marker for estimating genetic diversity of germplasm and different species depends on many factors including costs on genotyping. In recent years SSR's are increasingly being used for genotyping of cultivated and related wild species because SSR markers are more efficient in detection of polymorphism as they are multi allelic. The EST-SSR markers tested in this study should be very useful for introgression breeding including wild species.



L-Ladder. Sample 1-Indaf-5, 2- Indaf-7, 3- Indaf-8, 4- Indaf-9, 5- GPU-26, 6- GPU-28, 7- GPU-45, 8- GPU-48, 9- HR-911, 10-HR-374, 11- L-5, 12- MR-1, 13- MR-6, 14- ML-181, 15- ML-322, 16- PR-202, 17- PES-110, 18- PES-400, 19- RAU-8, 20- VR-708, 21- VL-149, 22- VL-305, 23- *Eleusine africana* and 24- *Eleusine indica*

Figure 1. Silver-stained sequencing gels showing polymorphic PCR products obtained using SSR primer UGEP 12, UGEP 8 and UGEP 73 in 24 finger millet genotypes.

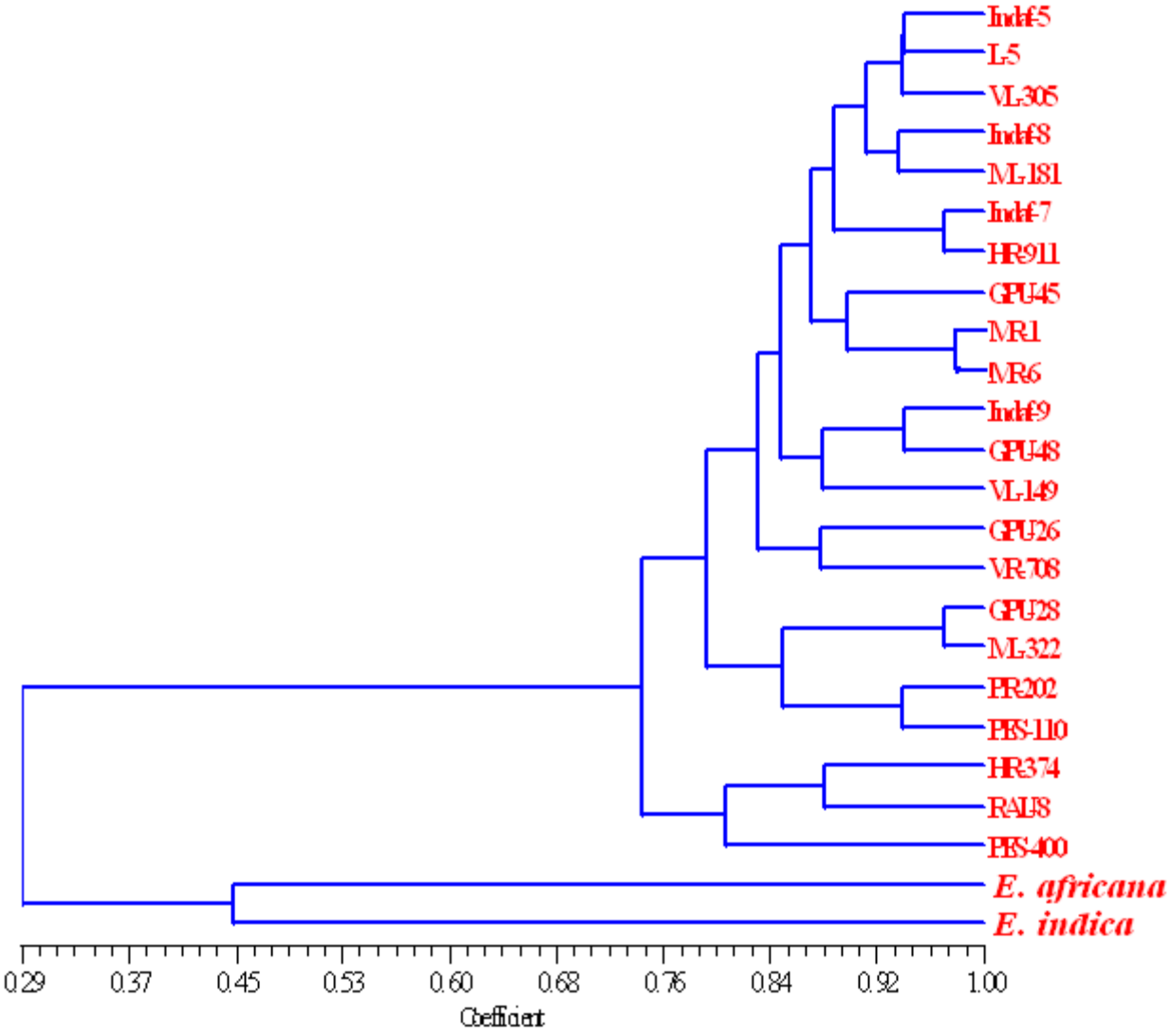


Figure 2. Dendrogram of 24 finger millet genotypes based on SSR markers run on PAGE using UPGMA analysis based on simple matching coefficient.

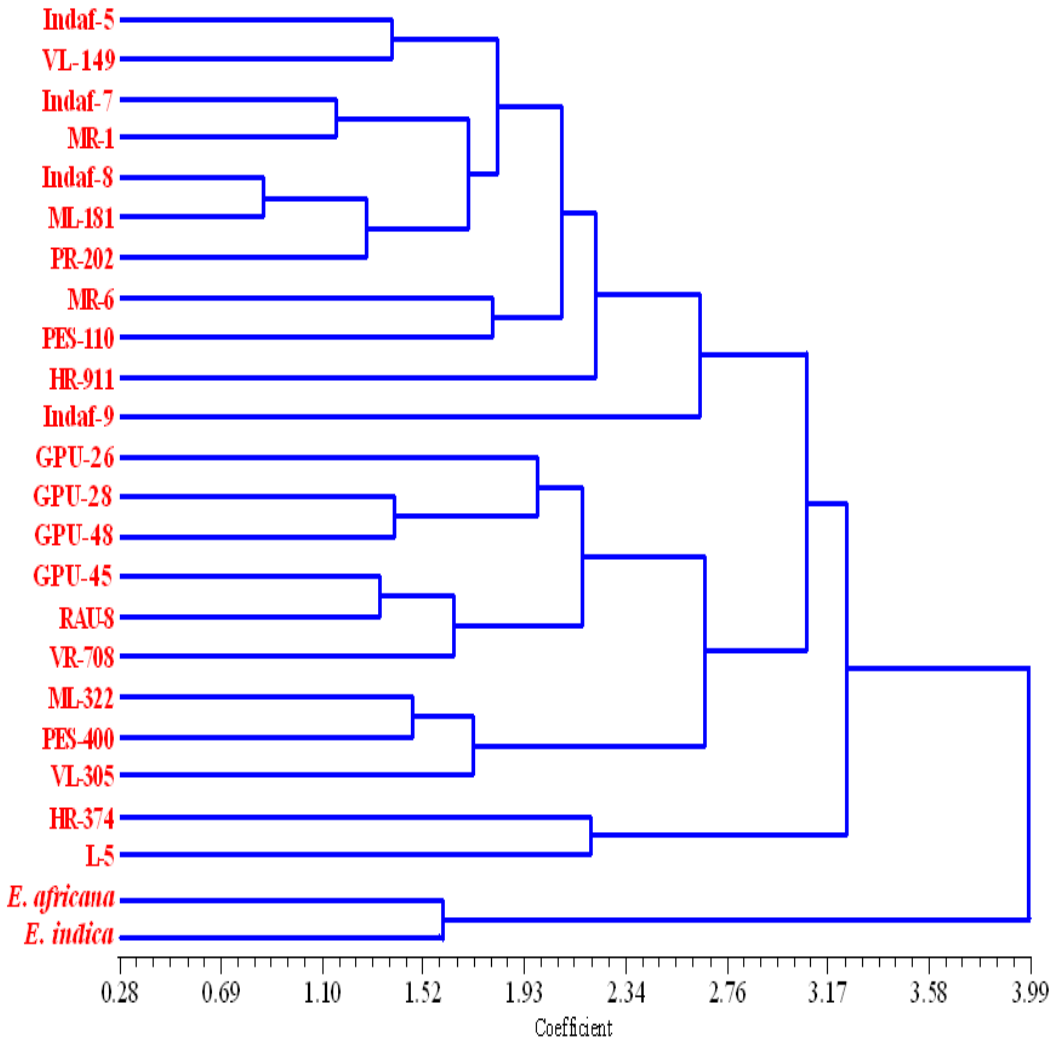


Figure 3. Dendrogram of 24 finger millet genotypes based on morphological data analyzed using the UPGMA generated by the Manhattan coefficient.

Table 4. Mean values of cultivated and wild species for 14 quantitative traits in finger millet.

Genotypes	N	Leaf Number	Flag leaf blade length (cm)	Flag leaf blade width (cm)	Flag leaf sheath length (cm)	Flag leaf sheath width (cm)	Peduncle length (cm)	Finger No.	Finger length (cm)	No. of days to 50 % flowering	Days taken to maturity	No. of productive tillers	Plant height at maturity (cm)	No. of grains/ spikelet	1000 seed weight (g)
Cultivated genotypes	22	11.85	36.52	1.13	13.01	0.76	24.10	8.11	7.71	6.35	104.90	4.32	97.95	4.56	2.58
Wild species	2	15.25	44.25	2.6	21.0	0.8	39.75	11.0	11.25	48.5	95.0	16.5	149.25	8.25	0.56
CD (0.05)		1.7	3.0	0.2	1.6	0.1	1.4	2.9	1.1	5	5	1.3	3.4	1.0	0.2

ACKNOWLEDGEMENTS

N. Nethra is highly grateful to the Kirkhouse Trust (U.K.) for providing the PhD fellowship.

REFERENCES

Anonymous (2009). AICSMIP – Quinquennial Review Report. ICAR, New Delhi pp. 1-50.

Barbeau WE, Hilu KW (1993). Protein, calcium, iron and amino acid content of selected wild and domesticated cultivars of finger millet. *Plant Foods Hum. Nutr.,* 43:97–104.

Bassam BJ, Caetana-Annoles G, Gresshoft PM (1991). Fast and sensistive silver staining of DNA in polyacrylamide gels. *Anal. Biochem,* 196: 80-83.

Collard BCY, Jahufer MZ, Brouwer JB, Pang, ECL (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for Crop Improvement: The basic concepts. *Euphytica* 42: 169-196.

Das S, Mishra RC, Rout GR, Aporajita S (2006). Genetic variability and relationships among the genotypes of finger millet (*Eleusine coracana* L. Gaertn) using RAPD markers. *Z. Naturforsch* 62: 116-122.

De Wet JMJ, Rao KEP, Brink DE, Mengesha MH (1984) Systamatics and evaluation of *Eleusine coracana* (Gramineae). *Am. J. Bot.,* 71: 550-557.

Dida MM, Srinivasachary, Ramakrishnan S, Bennetzen JL, Gale MD, Devos KM (2007). The genetic map of finger millet, Eleusine coracana. *Theor. Appl. Genet.* 114:321-332.

Dida MM, Wanyera N, Harrison Dunn ML, Bennetzen JL, Devos KM (2008). Population Structure and Diversity in Finger Millet (*Eleusine coracana*) Germplasm. *Trop. Plant Biol.,***5**: 21–32.

Fakrudin B, Kulkarni RS, Shashidhar HE, Shailaja H (2004). Genetic diversity assessment of finger millet, *Eleusine coracana* (Gaertn), germplasm through RAPD analysis. *Plant Genetic Resources* Newsletter, 138: 50-54.

FAO Stat Data (2011). FAO Statistical Databases (http://faostat.fao.org).

Gupta PK, Varshney RK (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica,* 113:163–185.

Harinarayana G, Seetharam A (1985). Descriptors for finger millet. IBPGR Secretariat, Rome.

Kottearachchi, NS (2013). Utility of DNA markers in rice breeding. *European International Journal of Science and Technology*, 2(8): 111-122.

Kuleung C, Baenziger PS, Dweikat I (2004). Transferability of SSR markers among wheat, rye and triticale. *Theor. Appl. Genet.* 108: 1147-1150.

Panse VG, Sukhatme PV (1961). Statistical methods for agricultural workers. *Indian Council of Agricultural research Publications*, New Delhi pg: 359.

Panwar P, Nath M, Yadav V, 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*, 2: 120-133.

Ramakrishna BM, Gowda BTS, Katti SM, Seetharam A, Mantur SG, Viswanath S, Krishnappa M, Viswanath KR, Krishnamurthy B, Jagadishwar K (1996). Evaluation of fingermillet germplasm. Project coordination cell, AICRP, GKVK, UAS, Bangalore, India.

Rohlf JF (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, NY.

Rongwen J, Akkaya AA, Bhagwat U, Lavi U, Cregan, PB (1995). The use of microsatellite DNA markers for soybean genotype identification. *Theor. Appl. Genet*. 90: 43-48.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984). Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci. USA* 83: 1757–1761.

Saghai-Maroof MA, Biyashev RM, Yang GP, Zhang Q, Allard RW (1994). Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. *Proc. Natl. Acad. Sci. USA* 91:5466–5470.

Sneath PHA, Sokal RR (1973). Numerical taxonomy. W.H. Freeman & Co., San Francisco.

Srinivasachary, Dida M, Gale MD, DevosKM (2007). Comparative analyses reveal high levels of conserved colinearity between the finger millet and rice genomes. *Theor. Appl. Genet.,* 115:489–499.

Sundaram GR, Venkatesam T, Vinod KK (2007). Genetic diversity among cultivars, land races and wild relatives of rice as revealed

by microsatellite markers. *J. Appl. Genet*. 48 (4): 337-345.

Vadivoo AS, Ritajoseph, Ganeshan NM, Joseph R, (1998). Genetic variability and diversity for protein and calcium contents in finger millet (*Eleusine coracana (L) Gaertn)* in relation to grain colour. *Plant Foods Hum. Nutr.*, 52(4): 353-364.

Varshney RK, Chabane K, Prasad S, Hendre, Ramesh K, Aggarwal, Graner A (2007). Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Science,* 173: 638–649.

Vogel JP, Tuna M, Budak H, Naxin Huo, Yong Gu Q, Steinwand MA (2009). Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon. BMC Plant Biology,* 9: 88.