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Research Article

Assessment of molecular diversity in an elite set of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes using SSR markers

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Abstract
Genetic diversity was assessed among 47 elite finger millet genotypes (29 varieties and 18 African and Asian germplasm) using 16 SSR markers. Owing to polyploidy nature of crop seven SSR markers have amplified more than one dose of alleles per genotype. Thirteen markers were polymorphic with polymorphic information content from 0.02 (UGEP56) to 0.61 (UGEP64) with an average of 0.22. A total of 72 alleles were detected across genotypes and alleles per SSR marker loci ranged from 1 to 14 (UGEP107) with an average of 4.69. UPGMA based dendrogram generated by Jaccard's similarity coefficient had grouped 47 genotypes in to three clusters and they were widely distributed on two dimension principle component analysis graph. Analysis of molecular variance revealed higher polymorphism in varieties (53.39 %) than in germplasm (39.71 %), implying higher genetic variability in cultivated varieties. The information gathered in the study will be useful in planning hybridization for further finger millet improvement.

Key words
Finger millet, molecular diversity, SSR marker, AMOVA, cluster

Introduction

Cultivated finger millet (*Eleusine coracana* subsp. *coracana*) is an allotetraploid (2n=4x=36) nutri-cereal crop species of the family *Poaceae* and sub-family *Chloridoideae* Srinivasachary *et al*. (2007). It has adapted to wide range of harsh eco-geographical conditions with minimal input and providing critical genetic resources for millions of poor farmers upon which they depend for staple food and livelihood income in infertile and marginal areas Hilu *et al*., (1979). It is the fourth most important millet covering 10 per cent of the global millet area in over 25 countries of Asia and Africa. India is the leading producer with an area of 1.13 million hectare and production of 1.82 million tonnes (MAFW, 2015-16) and major states growing finger millet are Karnataka, Tamil Nadu, Andhra Pradesh, Odisha, Maharashtra, Uttarakhand, Jharkhand and Gujarat. Nutritionally it is rich in dietary fibre, calcium, iron, manganese and methionine (Pragya and Rita, 2012), which are low or lacking in diets of millions who use rice as staple food. Because of its high nutritional value and excellent storage quality it is known as food security crop Dida *et al*.(2007). It has some medicinal properties also *viz*., hypoglycemic, hypocholestromic and is rich in antioxidants, hence highly valued for diabetics and for better gastrointestinal health Devi *et al*.(2011).

Assessment of genetic diversity of genotypes gives an idea to the breeder before attempting any hybridization program. Generally morphological traits are used in the assessment of genetic diversity, where phenotypic expression is invariably influenced by the environment. Use of Deoxyribonucleic Acid (DNA) markers which are environment insensitive would help in assessment of genetic variation at DNA level, which is more precise. In finger millet molecular diversity has been assessed using different marker systems *viz*., Random Amplified Polymorphic DNA (RAPD) Karad *et al.* (2013), Inter-Simple Sequence Repeat (ISSR) (Prabhu and Meenakshi 2013; Zuge *et al*. (2018), Simple Sequence Repeat (SSR) Prabhu and Meenakshi (2013); Nethra *et al.* (2014); Manyasa *et al*.(2015); Santie *et al*.(2015); Ramakrishnan *et al*.(2016); Gimode *et al.*(2016); Pandian *et al*.(2018) and Single Nucleotide Polymorphism (SNP) markers Kumar *et al*.(2016); Gimode,(2016).

Among different marker systems SSR marker is more appropriate for assessing genetic diversity because it

provides more informative molecular data compared to other marker techniques Stich *et al*. (2010). In finger miller several SSRs Dida *et al.*(2007); Gimode *et al.*(2016), Hittalmani *et al.(*2017), Expressed Sequence Tags (EST)-derived SSRs Babu *et al*., (2014); Obidiegwu *et al.(*2014); Pandian *et al*.(2018) and Single Nucleotide Polymorphism (SNP) markers Kumar *et al*.(2016); Gimode *et al*.(2016) have also been developed. Dida *et al*. (2007) have published 82 SSR markers from sequences of positive clones identified by hybridization in finger millet. Santie *et al*. (2015) analysed these markers for their usefulness in discerning genetic diversity by screening across 10 diverse genotypes and identified 20 informative SSR markers. Gimode *et al*. (2016) have sequenced two finger millet genotypes, KNE755 and KNE796 and identified 10,327 SSRs and 23,285 non-homologous SNPs. Validation of 101 SSRs and 92 SNPs across a set of genotypes for polymorphism resulted in significant polymorphic markers of 49 SSRs and 80 SNPs, respectively. Hittalmani *et al.* (2017) have sequenced whole genome of finger millet cultivar ML-365 using Illumina and SOLiD sequencing technologies, identified 114,083 SSRs and shortlisted 18,514 SSRs. Randomly chosen 35 SSRs validation in wet lab in ML-365 has confirmed the *in silico* e-PCR results and which were used for fingerprinting of 26 finger millet accessions, 14 wild species. Pandian *et al*. (2018) have developed 56 new genic SSR markers from publicly available drought related ESTs, of which 43 SSRs were polymorphic with PIC value from 0.41 to 0.79.

Use of SSR marker poses some problems in polyploids especially allopolyploids, mainly because of inability to identify/score homologous *vs.* homeologous alleles and therefore to estimate other primary statistics for (hetero)zygosity Huang *et al.* (2008) between and across ploidy. Several methods have been proposed to render microsatellites suitable for polyploid species Samadi *et al*. (1999); Espinoza & Noor (2002); Bruvo *et al*. (2004); Babaei *et al*. (2007). Non-availability of genetic resources for differentiating alleles of a locus in finger millet, individual alleles of each locus, in similar way as in previous studies, were scored as presence/absence (binary matrix) and used in calculation of similarity indices as in dominant markers. In the present study, a set of 16 SSR markers Dida *et al*.(2007) were used to assess genetic diversity among 47 selected finger millet genotypes.

Material and Methods

A total of 47 genotypes, that includes 29 improved varieties and 18 germplasm lines were collected from Project Coordinating Unit (Small millets), All India Coordinated Research project on Small Millets (AICRP-SM), Bengaluru, India. For this study, improved varieties from finger millet growing states, having special features like drought tolerance, blast-resistance, dwarfness, early flowering, non-lodging, high tillering, high protein content, adaptation to hilly and coastal belts (Table 1) were selected. Likewise, germplasm covering major finger millet growing countries of Africa and South Asia (mostly from India) having special features *viz*. high biomass content, high test weight, more productive tillers, blast disease resistance, high protein and calcium content along with high yielding nature were included for this study. Details of genotypes, their region/state of origin (varieties), continent (germplasm) and their features are listed in Table 1.

Genomic DNA was isolated from young leaves of 14 days old seedlings by following modified CTAB method Mace *et al*.,(2003).

The 47 genotypes were subjected to genotyping using 16 published SSR markers (Table 2) for finger millet Dida *et al*.(2007). In all forward primers, M13-tag (5′-CACGACGTTGTAAAACGAC-3′) was attached on 5′ end that allowed incorporation of a fluorescent label during PCR to facilitate detection of amplified products Schuelke (2000). PCR reaction was carried in 10 µL in 96 well microtitre plates and each reaction mixture comprised of Taq buffer (20 mMTris–HCl, pH 7.6; 100 mMKCl; 0.1 mM EDTA; 1 mM DTT; 0.5 per cent (w/v) Triton X-100; 50 per cent (v/v) glycerol), 2 mM $MgCl_2$, 2 mM dNTPs, 0.16 µM fluorescent labelled M13-forward primer, forward primer, 0.2 µM reverse primer, 0.2 units of Taq DNA polymerase (SibEnzyme Ltd, Russia) and 30 ng of template DNA. PCR reactions were carried out by following touchdown PCR program with an initial denaturation of 94°C for 5 min, followed by 10 cycles of denaturation at 94°C for 20 s, annealing temperature starting at 61°C for 20 s decreasing by 1°C/cycle and extension at 72°C for 20 s. Further this was followed by 35 cycles with an annealing temperature of 58°C for 20 s and final elongation at 72°C for 20 min. Amplification was confirmed by running 2 µL of the PCR products on a 2 % (w/v) agarose gel stained with ethidium bromide and visualised under UV light.

For the electrophoresis amplified products (1 µL–3.5 µL of each) of two SSR markers labelled with two different fluorochrome (FAM (blue) and VIC (green)) dyes (Applied Biosystems) were co-loaded together with an internal size standard, GeneScan™–

500 LIZ® (Applied Biosystems) and Hi-Di™Formamide (Applied Biosystems). Amplified products were separated by capillary electrophoresis using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems) and which has detected alleles differed by only few bases among the genotypes screened.

The allele calling was performed based on peak patterns in electropherogram (Fig. 1) across 47 genotypes using Gene Mapper 4.0 (Applied Biosystems). Out of 16 SSR markers nine markers recorded single dose allele amplification (single peak pattern per genotype as depicted in Fig. 1a) across 47 genotypes, however remaining seven SSR markers have exhibited more than one dose allele amplification (more than one peak pattern per genotype as depicted in Fig.1b & 1c) across 47 genotypes. Among them five SSR markers, UGEP33, UGEP96, UGEP3, UGEP79 and UGEP65 amplified two doses of alleles; while UGEP110 and UGEP107 SSR markers amplified three and seven dose of alleles (Fig.1c) per genotype, respectively. In total 16 SSR markers recorded 29 dose of putative alleles (including homeologus alleles) across 47 genotypes.

The raw allele sizes (in base pairs, bp) of 29 alleles were assigned to their appropriate allele-size 'bin', based on the microsatellite repeat length using Allelobin v2.0. Idury and Cardon, (1997). The 'binned' data set was used for analyzing genetic diversity parameters *viz.*, gene diversity Weir, (1996), Polymorphic Information Content PIC; Botstein *et al*. (1980), major allele frequency Weir, (1996), number of alleles per loci and availability and Analysis of MOlecular VAriances (AMOVA) using PowerMarker v.3.25 (Liu and Muse, 2005). The SSR marker which recorded multiple doses of alleles per genotype, genetic parameters (gene diversity, PIC, major allele frequency and availability) were estimated separately for each dose of allele and averaged out per SSR locus to get respective SSR marker value. A total number of alleles detected across all the allelic doses of SSR marker were taken as number of alleles per SSR marker locus. Gene diversity Weir(1996), Polymorphism Information Content PIC; Botstein *et al*. (1980) and Allele Frequency Weir, (1996) were also estimated.

A total of 72 alleles were detected from 16 SSR markers, where each allele was scored for its presence (1) and absence (0) across 47 finger millet genotypes like for dominant markers. This binary data was subjected for the construction of Jaccard's similarity matrix Jaccard(1901), which was subsequently used in the construction of hierarchical dendrogram based on UPGMA (Unweigh ted Pair Group Method using arithmetic Averages) algorithm and two dimension Principal Component Analysis (PCA) graph using NTSYSpc 2.02i (Rohlf, 1992).

Results and Discussion

All the 16 SSR markers revealed amplification in all the genotypes and out of which, nine markers *viz*., UGEP56, UGEP66, UGEP68, UGEP46, UGEP81, UGEP31, UGEP64, UGEP102 and UGEP95_2 recorded single allele (band) dose per genotype, UGEP33, UGEP96, UGEP3, UGEP79 and UGEP65 showed two allele (bands) dose, UGEP110 and UGEP107 recorded three alleles (bands) and seven alleles (bands) per genotype (Fig. 1c), respectively. The amplification of SSR marker at more than one allele dose could be attributed to homeologous loci of two genomes in finger millet Hiremath and Salimath, (1992), where segments of chromosomes might be present in more than two copies over genomes. Manyasa *et al*. (2015) also observed amplification of duplicate loci for UGEP110 marker, which were scored as two separate markers *viz*., UGEP110 and UGEP110-1. Similarly Santie *et al*. (2015) also observed two duplicate loci each for four markers *viz*., UGEP5, UGEP51, UGEP95 and UGEP103 and were scored as separate markers. A total 72 alleles were observed from 16 SSR markers, of which 63 alleles were polymorphic and 9 alleles were monomorphic. The allelic size among markers ranged from 153 bp (UGEP107 and UGEP102) to 261 bp (UGEP31). The number of alleles per SSR marker ranged from 1 (UGEP96, UGEP81 and UGEP95_2) to 14 (UGEP107) with an average of 4.69 allele per SSR marker. Similar allelic behaviour was reported by Nirgude *et al.* (2014), where allele range was from 2 to 8 with an average of 4.8 alleles per loci across 103 finger millet genotypes. Santie *et al*. (2015) has observed 14 alleles for UGEP24 marker across 10 diverse finger millet genotypes. More number of alleles per marker loci across genotypes suggests broader genetic base in the material.

Genetic diversity among finger millet genotypes was significant as indicated by PIC values and gene diversity values of the SSR markers. PIC values of 13 polymorphic SSR marker ranged from 0.02 (UGEP56) to 0.61 (UGEP64) with an average of 0.22 (Table 3). The highest PIC value was observed in UGEP64 (0.61) followed by UGEP33 (0.44) and UGEP66 (0.41). Similarly, gene diversity among finger millet genotypes varied from 0.02 (UGEP56) to 0.67 (UGEP64) with an average of 0.26 (Table 3); and highest gene diversity value was observed for UGEP64 (0.67) followed by UGEP31 (0.49),

UGEP33 (0.48), UGEP68 (0.47), UGEP66 (0.46) and UGEP46 (0.46). The mean PIC value (0.22) is comparatively less than 0.34 as reported by Nethra *et al* (2014) and mean genetic diversity value (0.26) across 47 genotypes were comparable with 0.33 as reported by Dida *et al*. (2008) among 79 finger millet accessions from Africa.

The UPGMA based dendrogram grouped 47 genotypes into three major clusters (Fig. 2). Cluster 2 was largest, with 28 genotypes, and was subdivided into three sub clusters 2A (16), 2B (8) and 2C (4). Cluster 1 was the second largest having 14 genotypes followed by Cluster 3 having 5 genotypes. The cluster 1 was dominated by varieties and cluster 3 by African germplasm, while cluster 2 was composed of both germplasm and varieties.

Clustering of genotypes derived from same geographical region was observed in UPGMA based dendrogram. Majority of genotypes from Andhra Pradesh (VR 762, VR 847, PR 1044) had fallen in sub cluster 2A; majority of genotypes from Karnataka had grouped in cluster 1 (HR 911, MR6, Indaf 7, L 5, Indaf 8, GE 1, and Indaf 5) and sub cluster 2B (GPU 67, GPU 28, GPU 66 and Indaf 9); genotypes from Uttarakhanda in cluster 1 (VL 315 and VL 149) and genotypes from Tamil Nadu in cluster 1 (CO 13 and GE 436). Among African genotypes, separate clustering was observed for Uganda (GE 4687, GE 4683 and GE 4693 in sub cluster 2A) and Kenya (GE 4764 and GE 2816 in cluster 3). Remaining germplasm from African and Asian continent and varieties from different states did not reveal any specific clustering pattern according to their geographical origin.

Majority of these genotypes consisted varieties selected from breeding programs, where selection pressure had invariably operated for adaptation to local condition, for example varieties from Andra Pradesh are adapted to costal climatic conditions, leading to fixation of common adaptation alleles among different varieties of same geographical region. Similarly clustering of African genotypes could be attributed to adaptability to their specific agro-climatic region. Interestingly clustering was noticed for some genotypes that share common features *viz*., non-lodging (GPU 67 and A 404 in sub cluster 2B), late duration (KOPN 235, MR 6, Indaf 7, GE 4972, GE 1 and Indaf 8 in cluster 1; GE 4764, GE 4939, GE 2816, GE 4906 and GE 5078 in cluster 3), high seed test weight (GE 4683 and GE 4693 in sub cluster 2A), adaptation to coastal region (VR 762, VR 847, PR 1044 in sub cluster 2A) and high grain yield (KOPN 235, HR 911, MR 6, Indaf 7, GE 4972, L 5, Indaf 8, GE 1 and Indaf 5, in cluster 1; GPU 67, GE 5038, GPU 28, GPU 66 and Indaf 9 in sub cluster 2B). The genotypes (GPU 66, PR 202 and GPU 28) related in the pedigree also showed clustering in sub cluster 2B, where GPU 66 is derived from the cross, PR 202 x GPU 28. The virescence mutant (GE 1) and its wild type (Indaf 8) were clustered together in cluster 1, which differed only for mutant gene(s). Clustering of genotypes according to geographical origin in finger millet was also reported by Dida *et al*. (2008), Bharathi (2011) and Babu *et al*. (2014). Babu *et al*. (2014) grouped 190 finger millet genotypes into two major clusters based on their geographical origin using 46 genomic SSR loci.

Principal Component Analysis (PCA) plot of first two dimensions have displayed interrelationship among 47 finger millet genotypes under study. First and second dimensions have accounted for 16.34 % and 13.83 % of the variance of SSR scored data, respectively, giving a cumulative variance of 30.17 % and their corresponding Eigen values were 2.89 and 2.45. Both germplasm and varieties appeared widely over two dimension plot of PCA (Fig. 3), indicating diverse nature of genotypes. Majority of genotypes revealed clustering pattern based on geographical origin and common features on PCA plot as observed in UPGMA dendrogram (Fig. 2).

Analysis of molecular variance (AMOVA) was performed utilizing 20 polymorphic SSR marker bands to partition total genetic variation into within and between germplasm and varieties (Table 4). AMOVA showed 93.10 % of total SSR allelic variation for within the group of germplasm and varieties, and 4.14 % between germplasm and varieties; implying comparatively less differentiation as groups between germplasm and varieties. Similarly, Ramakrishnan *et al*. (2016) have reported highest molecular variance for within population (93 %), lowest for among various geographical regions (4 %) and among populations (3 %). Between germplasm and variety groups, varieties (53.39%) have shown more genetic polymorphism over germplasm (39.71 %). Considerable genetic diversity within cultivated varieties could be due to the operation of continuous selection pressure (both artificial and natural) in different agro-climatic zones for adaptation and breeding selection on varieties for diverse special features. However, slightly less genetic variation within germplasm could be attributed to smaller number of germplasm (18) as

compared to varieties (29) besides stringent selection process in selecting the germplasm.

The analyses of genetic diversity and AMOVA have confirmed the existence of significant genetic variation among all the genotypes and within varieties and germplasm. UPGMA based cluster analysis showed the clustering of genotypes according to their geographical origin, their common characteristics features and pedigree relationship. Many of the test genotypes have been used frequently in hybridization breeding and hence diversity information among these elite genotypes could be useful in selection of parents in breeding program.

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Table 1. List of finger millet genotypes along with their origins state/continent.

Serial Number	Variety	State/continent of origin	Salient features	Serial Number	Variety/ germplasm	State/continent of origin	Salient features
1	GPU 28	Karnataka (India)	High yielding, blast resistant	25	A 404	Jharkhand (India)	Drought tolerant, deep root system, dwarf, non-lodging
2	GPU 66	Karnataka (India)	Green plant parts with narrow leaves	26	BM 2	Jharkhand (India)	Tolerant to drought, moderately blast resistant
3	GPU 67	Karnataka (India)	High tillering, non-lodging	27	GN 2	Gujarat (India)	Drought tolerant, high finger number
4	Indaf 5	Karnataka (India)	High yielding	28	OEB 526	Odisha (India)	Non-lodging, moderately blast resistant
5	Indaf 7	Karnataka (India)	Cold tolerant	29	KOPN 235	Maharastra (India)	Suitable for sub mountain and Ghats zone
6	Indaf 8	Karnataka (India)	High yielding	30	GE 4687	Africa (Uganda)	More productive tillers
7	Indaf 9	Karnataka (India)	Early maturity	31	GE 4693	Africa (Uganda)	High test weight & grain yield
8	MR1	Karnataka (India)	High yielding	32	GE 4683	Africa (Uganda)	High biomass content & test weight
9	MR 6	Karnataka (India)	High yielding, drought tolerant	33	GE 4972	Africa (Zambia)	More productive tillers and grain yield
10	L 5	Karnataka (India)	Blast resistant	34	GE 4939	Africa (Zambia)	High grain yield
11	HR 911	Karnataka (India)	High yielding	35	GE 5038	Africa (Zimbabwe)	High yield and ear weight
12	Uduru Mallige	Karnataka (India)	Early duration, dwarf	36	GE 5078	Africa (Zimbabwe)	More finger numbers
13	VR 762	Andra Pradesh (India)	Moderately blast resistant	37	GE 4798	Africa (Kenya)	High yielding and high biomass
14	VR 847	Andra Pradesh (India)	Moderately blast resistant	38	GE 2816	Africa (Kenya)	Highly heat tolerant
15	PPR 2350	Andra Pradesh (India)	suited to coastal area	39	GE 4764	Africa (Kenya)	High biomass content
16	PR 202	Andra Pradesh (India)	Stay green, wide adaptation	40	GE 3112	Africa (Malawi)	High Ca & protein content
17	PR 1044	Andra Pradesh (India)	Drought tolerant, protein rich	41	GE 4703	Africa (Ethiopia)	More productive tillers
18	CO 10	Tamil Nadu (India)	Dwarf, stay green, protein rich	42	GE 4906	Africa	Small glumes, naked seeds
19	CO 13	Tamil Nadu (India)	Non-lodging	43	GE 4449	Asia (India)	Blast resistant
20	CO 14	Tamil Nadu (India)	Moderately resistant blast	44	GE 1130	Asia (India, Uttar Pradesh)	High harvest Index and grain yield
21	TRY 1	Tamil Nadu (India)	Dual purpose, Salinity tolerant	45	GE 1	Asia (India, Karnataka)	Virescent mutant of Indaf 8
22	PRM 1	Uttarakhanda (India)	Early, dwarf and adapted to hilly region	46	GE 436	Asia (India, Tamil Nadu)	Drought tolerant, blast resistant
23	VL 315	Uttarakhanda (India)	Early duration, dwarf	47	GE 3666	Asia (India, Maharashtra)	More productive tillers, dwarf
24	VL 149	Uttarakhanda (India)	Earliness, blast resistant				

Table 2. Primer sequences, SSR repeat length, and PCR product length of SSR markers (Dida *et al.*, 2007) used for assessing genetic diversity among parental lines used in the study

Serial Number	Primer	Primer sequence	SSR motif	Product length (bp)	LG*
1	UGEP110F	AAATTC*GCA*TCCTTGCTGAC	$(CT)_{12}$	192	-
	UGEP110R	TGACAAGA*GCA*CACCGACTC			
2	UGEP33F	TAGCCCGTTTGCTTGTTTTG	$(TC)_{18}$	216	-
	UGEP33R	AAGGCCCTAGAACGTCAAGC			
3	UGEP96F	TAATGGGCCTAATG*GCA*ATG	$(CT)_{10}$	211	9A
	UGEP96R	CAAAATCCGAGCCAAGATTC			
4	UGEP56F	CTCCGATACAGGCGTAAAGG	$(GT)_{12}$	162	-
	UGEP56R	ACCATAATAGGGCCGCTTG			
5	UGEP3F	CCACGAGGCCATACTGAATAG	$(CA)_7N_{12}(GA)_{15}$	206	3AbB
	UGEP3R	GATGGCCACTAGGGATGTTG			
6	UGEP66F	CAGATCTGGGTAGGGCTGTC	$(AG)_{29}$	219	-
	UGEP66R	GATGGTGGTTCATGCCAAC			
7	UGEP68F	CGGTCA*GCA*TATAACGAATGG	$(CT)_{14}$	232	9B
	UGEP68R	TCATTGATGAATCCGACGTG			
8	UGEP46F	CAAGTCAAAACATTCAGATGG	$(GA)_{14}$	163	-
	UGEP46R	CCACTCCATTGTAGCGAAAC			
9	UGEP107F	TCATGCTCCATGAAGAGTGTG	$(GA)_{15}$	224	-
	UGEP107R	TGTCAAAAACCGGATCCAAG			
10	UGEP79F	CCACTTTGCCGCTTGATTAG	$(CT)_{12}$	173	-
	UGEP79R	TGACATGAGAAGTGCCTTGC			
11	UGEP81F	AAGGGCCATACCAACACTCC	$(GT)_{12}$	192	6B
	UGEP81R	CACTCGAGAACCGACCTTTG			
12	UGEP65F	AGTGCTAGCTTCCCATCAGC	$(CT)_{19}$	226	-
	UGEP65R	ACCGAAACCCTTGTCAGTTC			
13	UGEP31F	ATGTTGATAGCCGGAAATGG	$(GA)_{12}$	241	-
	UGEP31R	CCGTGAGCCTCGAGTTTTAG			
14	UGEP64F	GTCACGTCGATTGGAGTGTG	$(CT)_{23}$	206	-
	UGEP64R	TCTCACGT*GCA*TTTAGTCATTG			
15	UGEP102F	AT*GCA*GCCTTTGTCATCTCC	$(TG)_{17}$	184	10
	UGEP102R	GATGCCTTCCTTCCCTTCTC			
16	UGEP95_2F	AGGGGACGCTTGGTTATTTG	$(TC)_{14}$	208	-
	UGEP95_2R	GCCTCTACCTGTCTCCGTTG			

bp, base pairs; *LG, Linkage group where marker has been mapped (Dida *et al*., 2007).

Table 3. Characteristics of 16 SSR markers screened across 47 finger millet genotypes.

Serial Number	Marker	Major Allele frequency	Amplified bands / genotype	Allele number/ loci	Allele size range (bp)	Availability	Gene Diversity	PIC
1	UGEP110	0.73	3	8	173-217	0.87	0.35	0.28
2	UGEP33	0.66	2	8	208-234	0.94	0.48	0.44
3	UGEP 96	1.00	2	1	209	1.00	0.00	0.00
4	UGEP56	0.99	1	2	182-196	1.00	0.02	0.02
5	UGEP3	0.91	2	6	203-227	1.00	0.16	0.15
6	UGEP66	0.69	1	5	183-233	0.89	0.46	0.41
7	UGEP68	0.65	1	3	251-257	0.91	0.47	0.38
8	UGEP46	0.68	1	4	181-189	1.00	0.46	0.39
9	UGEP107	0.92	7	14	153-245	0.87	0.11	0.09
10	UGEP79	0.97	2	2	188-192	0.81	0.05	0.05
11	UGEP81	1.00	1	1	196	0.96	0.00	0.00
12	UGEP 65	0.73	2	8	214-258	1.00	0.32	0.29
13	UGEP31	0.61	1	3	257-261	1.00	0.49	0.39
14	UGEP64	0.46	1	6	170-182	1.00	0.67	0.61
15	UGEP102	0.95	1	3	153-161	0.83	0.10	0.10
16	UGEP95_2	1.00	1	1	159	0.77	0.00	0.00
	Mean	0.81		4.69		0.93	0.26	0.22

bp, base pairs; PIC, Polymorphic Information Content.

Table 4. Analysis of molecular variance for two groups of finger millet genotypes (germplasm and variety).

Source	Sum of Square	Percentage
Among Populations	23.98	4.14
Within Population (Germplasm)	229.81	39.71
Within Population (Variety)	308.98	53.39
Within Individuals (Germplasm)	14.00	2.42
Within Individuals (Variety)	2.00	0.35
Total	578.76	100.00

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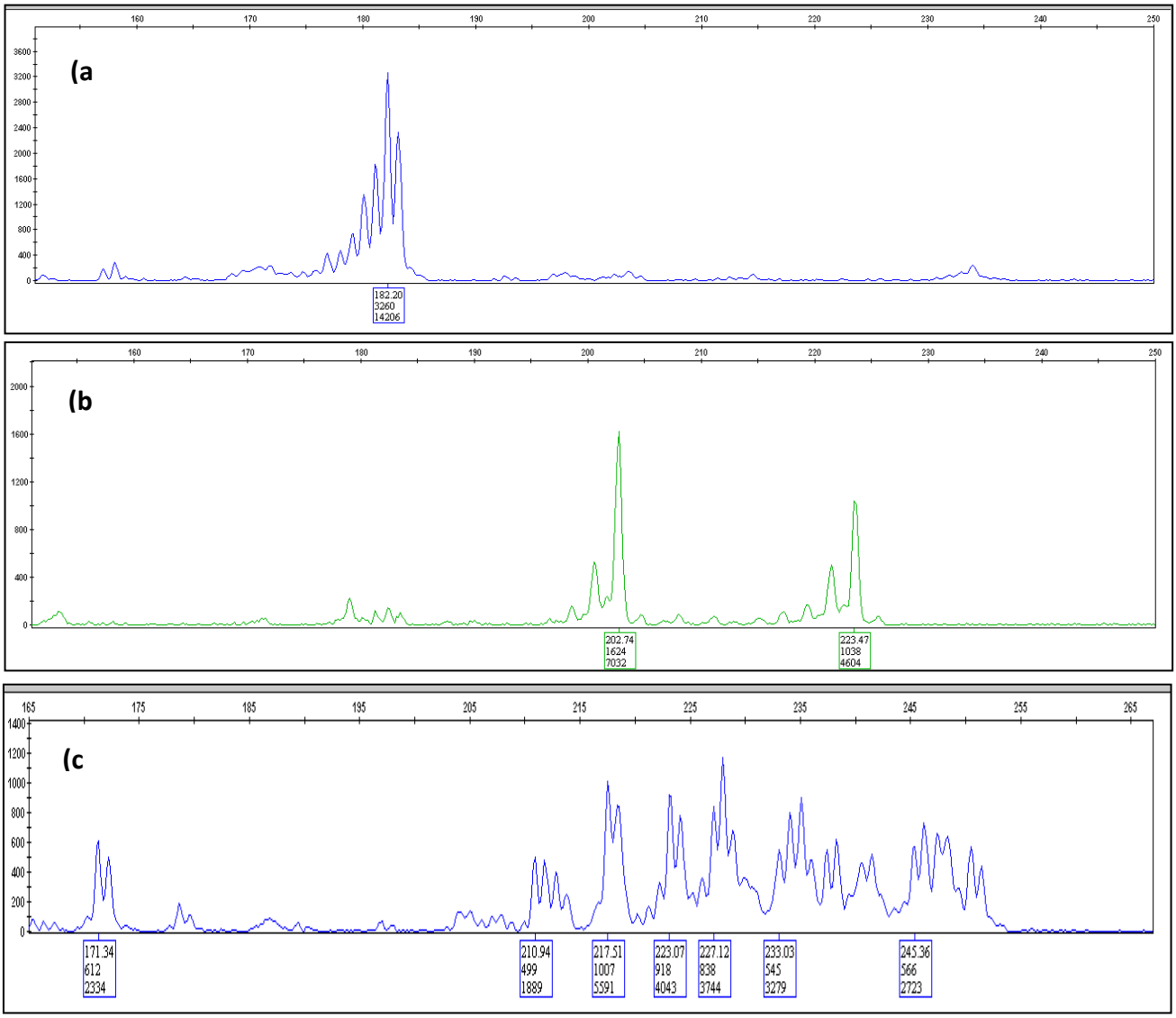

Fig. 1. Electropherogram of SSR markers showing peak pattern(s) in finger millet genotype. In each of 47 finger millet genotypes UGEP56 marker amplified one band (a), UGEP3 marker amplified two bands (b) and UGEP107 marker amplified seven bands (c). The peak patterns of these markers were similar in all the 47 genotypes, which were manually scored as allele size in base pairs (indicated below peak pattern along with peak intensity).

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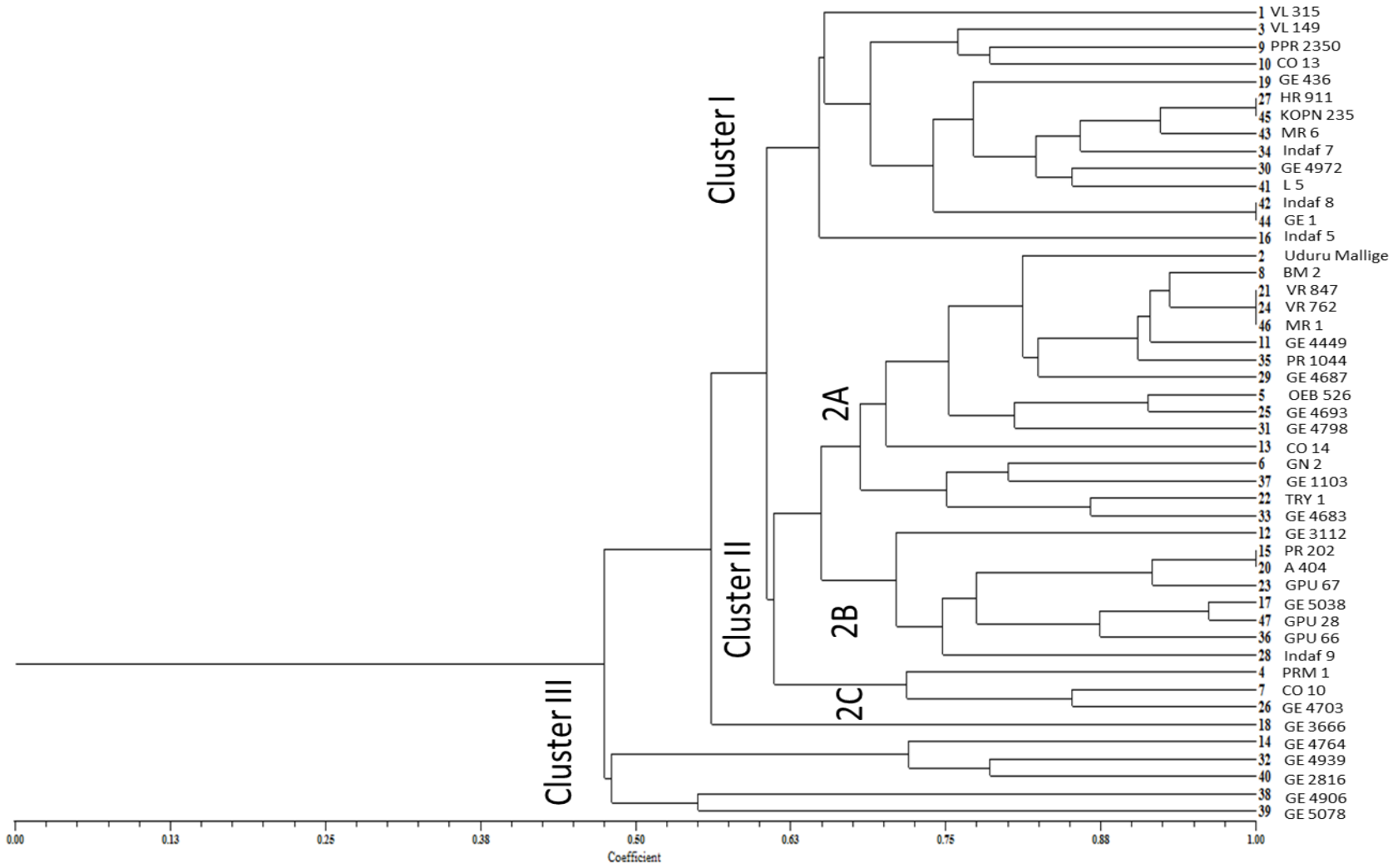

Fig. 2. UPGMA based dendrogram generated by Jaccard's similarity coefficient using 16 SSR markers depicting genetic relationship among 47 finger millet genotypes derived from different parts of world. Forty seven genotypes are grouped into three major clusters, cluster 2 is the largest, subdivided into three sub clusters (2A, 2B and 2C).

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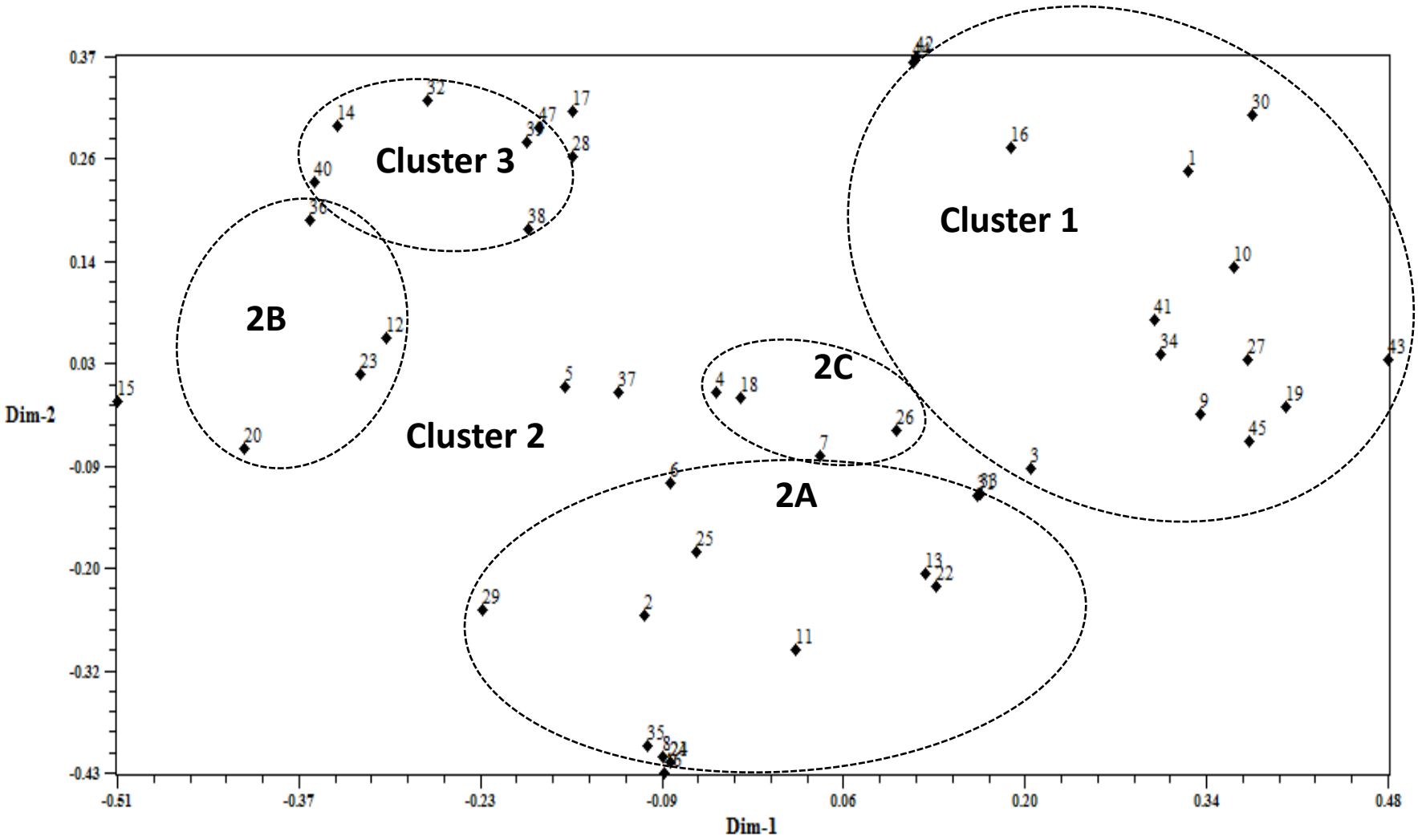

Fig. 3. Two dimensional Principal Component Analysis graph showing distribution of 47 finger millet genotypes. Majority of genotypes have followed similar clustering pattern (dotted circle) as in UPGMA dendrogram.