

## Population Structure and Genetic Variation in Indian and African *Eleusine coracana* (L.) Gaertn

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Population structure and genetic variation was estimated for 40 Indian and African finger millet genotypes using 237 random amplified polymorphic DNA (RAPD) and 16 morphological markers revealing differences between African and Indian groups. African genotypes showed 53.59%; 0.17±0.19; 0.27±0.27 compared to 48.10%; 0.15±0.19; 0.24±0.27 polymorphism, Nei's gene diversity and Shannon's Information index, respectively in Indian genotypes. Primers OPM-20, OPW-04, OPX-11 were identified as the most informative primers in terms of high % polymorphism and Nei's gene diversity values. Population structure analysis revealed the existence of two distinct sub-groups, correlated well with primary and secondary centers of finger millet diversity and can be utilized to enhance and widen finger millet germplasm base.

**Key Words: Finger millet, RAPD, Genetic diversity, Population structure**

### Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn., family Poaceae (Gramineae), subfamily Chloridoideae and tribe Eragrostideae; 2n=4x=36; genomic constitution AABB) is a subsistence food crop with excellent storage qualities and has high levels of calcium, iron, methionine-amino acid rich protein, soluble fibre and high diastatic power of malted grains (Barbeau and Hilu, 1993; Yenagi *et al.*, 2010). Cultivated finger millet was domesticated some 5000 years ago in African highlands and moved to Indian subcontinent around 3000 B.C. There is vast scope for improving cultivars by intercrossing of African with Indian germplasm leading to good value addition to this crop. Evaluation of genotypic variation of Indian and African germplasm based on molecular markers along with its phenotypic characterization would play a vital role in selecting diverse parents for finger millet breeding programmes. Reports available on assessment of genetic diversity/population structure among finger millet genotypes at molecular level till date pertains to RAPD, SSR, cytochrome P450 gene based marker systems (Fakrudin *et al.*, 2004; Babu *et al.*, 2007; Dida *et al.*, 2008; Panwar *et al.*, 2010; Arya *et al.*, 2013). The objective of this study was to characterize a set of 40 finger millet genotypes originating from seven African countries and seven different states of India based on

morphological and RAPD (RAPDs are valuable in terms of their high genomic abundance and random distribution throughout the genome) markers. Further, population sub-structure and partitioning of genetic variation was discussed in order to emphasize the utility of studied material in finger millet crop improvement.

### Materials and Methods

#### Plant Materials

For the current study, the seeds of 40 diverse finger millet genotypes (Table 1) were procured from All India Coordinated Small Millet Improvement Project (AICSMIP), Bangalore, India.

#### Evaluation of Phenotypic Diversity

The selected finger millet genotypes were evaluated in Augmented Block Design for their agronomic performance at AICSMIP, Bangalore, India. Data were recorded for 16 morphological traits (Table 2).

#### DNA Extraction and PCR Amplification

Extraction of total genomic DNA was carried out following cetyl-trimethyl-ammonium bromide (CTAB) method (Saghai-Marouf *et al.*, 1984) with minor modifications. DNA was extracted from bulk leaf samples (30 individual plants/sample) of 6-week-old

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**Table 1. Forty finger millet genotypes used in the study and their sub-population membership coefficient**

GE No.	Origin	G1	G2
GE28	Tamil Nadu, India	0.052	0.948
GE29	Tamil Nadu, India	0.026	0.974
GE62	Tamil Nadu, India	0.161	0.839
GE202	Tamil Nadu, India	0.075	0.925
GE595	Tamil Nadu, India	0.018	0.982
GE753	Tamil Nadu, India	0.017	0.983
GE1736	Tamil Nadu, India	0.010	0.990
GE1801	Tamil Nadu, India	0.010	0.990
GE174	Andhra Pradesh, India	0.053	0.947
GE314	Andhra Pradesh, India	0.022	0.978
GE617	Andhra Pradesh, India	0.017	0.983
GE1361	Andhra Pradesh, India	0.013	0.987
GE918	Karnataka, India	0.047	0.953
GE580	Bihar, India	0.022	0.978
GE1360	Andhra Pradesh, India	0.008	0.992
GE68	Uttaranchal, India	0.088	0.912
GE894	Uttaranchal, India	0.014	0.986
GE1924	Uttaranchal, India	0.031	0.969
GE565	Madhya Pradesh, India	0.026	0.974
GE594	Madhya Pradesh, India	0.011	0.989
GE468	Kerala, India	0.021	0.979
GE4690	Uganda, Africa	0.251	0.749
GE4682	Uganda, Africa	0.210	0.790
GE4725	Kenya, Africa	0.647	0.353
GE4752	Kenya, Africa	0.947	0.053
GE4765	Kenya, Africa	0.607	0.393
GE4796	Kenya, Africa	0.687	0.313
GE5121	Kenya, Africa	0.182	0.818
GE5130	Kenya, Africa	0.978	0.022
GE4915	Zambia, Africa	0.983	0.017
GE4927	Zambia, Africa	0.973	0.027
GE4951	Zambia, Africa	0.146	0.854
GE5025	Zimbabwe, Africa	0.972	0.028
GE5027	Zimbabwe, Africa	0.043	0.957
GE5029	Zimbabwe, Africa	0.976	0.024
GE4990	Tanzania, Africa	0.115	0.885
GE4991	Tanzania, Africa	0.982	0.018
GE4813	Malawi, Africa	0.980	0.020
GE4833	Malawi, Africa	0.989	0.011
GE5013	Ethiopia, Africa	0.986	0.014

plant. DNA quantification was done using DyNA Quant 200 fluorometer (Hoefer Instruments, USA). A total of twenty five primers with well resolved and distinct banding pattern were used for RAPD amplification. For the PCR reaction, a final volume of 25  $\mu$ l was prepared. PCR amplification was carried out with 25 ng of genomic DNA, 3 mM MgCl<sub>2</sub> (Fermentas Life Sciences), 1U Taq DNA polymerase (Fermentas Life Sciences), 1x PCR buffer without MgCl<sub>2</sub> (Fermentas Life Sciences), 0.2  $\mu$ M decamer primers (Operon) and 0.2 mM of dNTP mix (Fermentas Life Sciences). PCR reactions were carried out in a Perkin Elmer GeneAmp PCR system 9600 thermocycler. PCR reaction cycles consisted of initial denaturation at 94°C for 5 min, followed by forty cycles of denaturation at 94°C for 1 min, primer annealing at 37°C for 1 min and primer extension at 72°C for 2 min and final extension step at 72°C for 10 min. The amplification products were loaded onto a 1.4% agarose gel and were recorded using a Bio Imaging System (SynGene).

### Statistical Analyses

The mean, range and standard deviation for each trait were calculated in order to identify which traits varied significantly between and within Indian and African subpopulations. RAPD fragments were scored visually, as absent (0) or present (1) and Rp (Resolving power) index was calculated as given by Prevost and Wilkinson (1999). POPGENE version 1.32 (Yeh *et al.*, 2000) was used to analyze gene diversity and population differentiation. Jaccard's similarity coefficient was calculated for UPGMA clustering using NTSYS-pc. ver. 2.1 (Rohlf, 2000). In addition, the software STRUCTURE (Pritchard *et al.*, 2000) was used to investigate population structure using a burn-in of 100,000, a run length of 10,00,000 (admixture model). The number of sub-groups/populations (K) was determined by running the program at different K values (1 to 6). Five independent runs were assessed for each K value. Number of distinct subpopulations was finally inferred based on maximum Ln P(D) and peak value of delta K (Evanno *et al.*, 2005).

### Results and Discussion

Average plant height, culm thickness, productive tillers, leaf number, flag leaf blade length, peduncle length, finger length, days to 50% flowering, days to maturity and grain yield (g) were higher across African as compared to Indian group (Table 2). Similar observations were also recorded by earlier workers (Dida *et al.*, 2008).

**Table 2. Mean, Range and Standard Deviation (SD) for the phenotypic traits analyzed**

Trait	African genotypes			Indian genotypes		
	Mean	Range	SD	Mean	Range	SD
Plant height (cm)	98.25	58-137	21.84	94.86	61-117	14.93
Culm thickness (cm)	1.01	0.8-1.3	0.13	0.82	0.6-1	0.11
Productive tillers	4.78	3.4-7.2	1.04	4.19	3.0-7	1.29
Leaf numbers	10.90	8.0-15	1.92	9.14	7.0-11	1.28
Flag leaf blade length (cm)	34.12	21.4-48.6	8.87	30.55	21.1-45.1	6.86
Flag leaf width (cm)	1.07	0.9-1.5	0.17	0.97	0.6-1.2	0.13
Leaf length (cm)	38.87	25.8-55.6	10.27	44.53	30-52.4	5.48
Leaf width (cm)	1.18	1.02-1.5	0.14	1.19	0.9-1.5	0.17
Peduncle length (cm)	24.56	19-30.8	3.48	24.00	18.0-32	3.77
Finger number	6.84	5.6-8.8	1.01	7.24	5.0-12	1.61
Finger length (cm)	6.83	4.7-9.8	1.38	5.95	3.7-8.8	1.48
Finger width (cm)	1.12	1.02-1.3	0.08	0.96	0.8-1.2	0.11
Days to 50 per cent flowering	68.75	63-73	3.49	58.00	46-68	6.43
Days to maturity	114.75	99-125	8.03	98.52	86-113	7.89
Test weight (g)	2.04	1.9-2.4	0.14	2.17	1.1-3.6	0.55
Grain yield (g)	20.01	9.1-33.0	6.50	10.48	2.0-19.0	4.61

Leaf length, finger number and test weight (g) was more for Indian as compared to African group in the tested location. Region wise data for these traits are not shown here. Mean values for leaf length and test weight was highest for genotypes from Tamil Nadu. Mean finger number was highest for genotypes from Uttaranchal. Mean culm thickness was highest in Kenyan genotypes. Genotypes from Malawi and Zambia showed longest mean value of days to 50% flowering and days to maturity respectively. Mean grain yield was highest for Zambian genotypes. And for rest of the traits Ugandan genotypes were showing highest mean values.

A total of 237 RAPD bands were amplified using 25 primers with a mean of 9.48 bands per primer, which was comparable to earlier studies (Babu *et al.* 2007, Panwar *et al.* 2010). Of the 237 RAPD fragments, 155 (65.4%) were polymorphic and the average number of polymorphic bands per primer was 6.2. The resolving power for the primer OPM-20 was 6.5 and was most informative followed by OPV-06 (4.61) and OPL-07 (4.35) in distinguishing individual genotypes. For twenty one Indian genotypes, a total of 229 bands were generated and 114 of them were polymorphic (48.10%) while African genotypes amplified 127 polymorphic (53.59%) out of 230 bands (Table 3). Our results indicated that African genotypes were showing relatively more polymorphism as compared to Indian genotypes, similar to previous reports (Fakrudin *et al.*, 2004; Dida *et al.*,

2008; Arya *et al.*, 2013).

The RAPD primers varied in their power to detect diversity within Indian and African genotypes. Some primers, such as OPM-20, OPW-04, OPX-11, revealed high diversity levels in both the African and Indian genotypes, whereas OPC-06 and OPH-12 detected very low variation across the two groups. The mean diversity value for all the 40 genotypes was  $0.18 \pm 0.18$  and Shannon's Information index ranged from 0.09 to 0.48 with a mean of  $0.29 \pm 0.25$ . Averaged over all the markers and genotypes, African genotypes displayed higher genetic variation ( $0.17 \pm 0.19$ ) as compared to Indian population ( $0.15 \pm 0.19$ ) and also higher mean Shannon's Information index for African ( $0.27 \pm 0.27$ ) as compared to Indian sub-population ( $0.24 \pm 0.27$ ). Lower diversity within Indian population as compared to African genotypes suggested that Indian sub-population arose from a relatively small number of founder plants (Dida *et al.*, 2008).

Region wise, genetic diversity parameters like percent of polymorphic loci, the mean observed number of alleles, the mean effective number of alleles, the mean Nei's gene diversity and mean Shannon's Information index are shown in Table 4. The results revealed that finger millet genotypes from Tamil Nadu region of the Indian subcontinent were most divergent as compared to other regions. In case of African sub-population, genotypes from Zambia followed by Kenya were most

**Table 3. Comparison of the level of polymorphism and diversity between Indian and African genotypes using RAPD markers**

Primer	Indian genotypes					African genotypes				
	TNB	NPB	P (%)	h	I	TNB	NPB	P (%)	h	I
OPB-07	9	4	44.44	0.17	0.26	9	1	11.10	0.03	0.05
OPB-08	8	2	25.00	0.06	0.10	9	4	44.44	0.09	0.16
OPB-18	14	10	71.40	0.21	0.32	13	9	69.20	0.19	0.29
OPC-06	5	1	20.00	0.07	0.11	5	1	20.00	0.03	0.05
OPD-08	14	7	50.00	0.19	0.28	14	7	50.00	0.16	0.25
OPD-13	6	4	66.66	0.14	0.23	6	3	50.00	0.14	0.21
OPF-14	5	2	40.00	0.13	0.20	5	4	80.00	0.33	0.49
OPF-20	8	4	50.00	0.14	0.22	8	3	37.50	0.15	0.23
OPG-02	8	5	62.50	0.18	0.28	8	6	75.00	0.27	0.40
OPH-12	10	4	40.00	0.07	0.11	9	2	22.20	0.04	0.06
OPH-19	8	3	37.50	0.12	0.18	9	6	66.66	0.27	0.40
OPL-01	8	3	37.50	0.15	0.22	8	4	50.00	0.16	0.24
OPL-07	15	11	73.30	0.23	0.34	14	10	71.40	0.19	0.30
OPM-05	11	3	27.20	0.07	0.11	11	6	54.50	0.20	0.30
OPM-07	9	4	44.44	0.15	0.23	9	6	66.66	0.18	0.29
OPM-18	8	4	50.00	0.13	0.20	9	5	55.50	0.16	0.25
OPM-20	14	12	85.75	0.31	0.46	14	11	78.50	0.29	0.42
OPV-06	13	7	53.80	0.21	0.31	12	8	66.66	0.23	0.34
OPV-07	12	3	25.00	0.08	0.12	14	5	35.70	0.13	0.19
OPW-04	9	6	66.66	0.23	0.35	9	8	88.80	0.22	0.36
OPW-19	7	2	28.50	0.09	0.14	7	3	42.80	0.17	0.25
OPW-20	7	3	42.80	0.12	0.19	7	3	42.80	0.19	0.27
OPX-11	5	4	80.00	0.23	0.37	5	4	80.00	0.23	0.36
OPX-14	10	3	30.00	0.09	0.14	10	3	30.00	0.08	0.13
OPX-17	6	3	50.00	0.10	0.17	6	5	83.30	0.23	0.38
	229	114	48.10	0.15	0.24	230	127	53.59	0.17	0.27

TNB= Total number of bands; NPB= Number of polymorphic bands; P(%)= % Polymorphism; h= Nei's gene diversity; I= Shannon's information index

**Table 4. Genetic diversity parameters of genotypes collected from diverse regions of India and Africa**

Region	% (P)	na	ne	h	I
Tamil Nadu	35.86	1.3586±0.4806	1.2626±0.3890	0.1449±0.2068	0.2101±0.2940
Andhra Pradesh	26.16	1.2616±0.4404	1.1924±0.3570	0.1057±0.1879	0.1535±0.2675
Uttaranchal	16.46	1.1646±0.3716	1.1214±0.2936	0.0676±0.1572	0.0983±0.2257
Madhya Pradesh	10.13	1.1013±0.3023	1.0716±0.2138	0.0419±0.1252	0.0612±0.1828
Uganda	13.08	1.1308±0.3379	1.0925±0.2389	0.0542±0.1400	0.0791±0.2043
Kenya	27.85	1.2785±0.4492	1.2172±0.3731	0.1183±0.1979	0.1701±0.2810
Zambia	32.49	1.3249±0.4693	1.2476±0.3858	0.1363±0.2036	0.1971±0.2903
Zimbabwe	22.36	1.2236±0.4176	1.1777±0.3509	0.0964±0.1846	0.1385±0.2624
Tanzania	19.83	1.1983±0.3996	1.1402±0.2825	0.0821±0.1655	0.1199±0.2416
Malawi	10.55	1.1055±0.3078	1.0746±0.2177	0.0437±0.1275	0.0638±0.1862

na= Observed number of alleles; ne= Effective number of alleles; h= Nei's gene diversity; I= Shannon's information index (Sample size was one for Karnataka, Bihar, Kerala and Ethiopia regions and hence not included for this analysis)

divergent. And genotypes from Madhya Pradesh and Malawi showed the lowest genetic variation. Greater genetic gains can be obtained by using these genotypes in finger millet crop improvement.

The Jaccard's similarity coefficients (JSC) values for RAPD markers ranged from 0.68 to 0.93 with a mean JSC value of 0.82. The present study identified GE 4927 (Zambia) and GE 62 (Tamil Nadu) as diverse genotypes, which were also found distinct on the basis of morphological traits like plant height (67.4 cm, 112 cm), productive tillers (4.2, 6), leaf length (31.6 cm, 50 cm), finger width (1.12 cm, 0.9 cm), peduncle length (19 cm, 32 cm), days to 50% flowering (71, 59) and days to maturity (119, 95) respectively. The mean value of JSC for the RAPD markers for Indian genotypes is 0.84 and 0.82 for African genotypes, thereby showing more variation in African as compared to Indian genotypes. The Jaccard's similarity based dendrogram was also constructed for the RAPD markers and is depicted in Fig. 1. The forty genotypes were divided into two groups.

Group I consists of five sub-groups (IA, IB, IC, ID and IE) that includes all the 21 Indian and 11 African (8 are in sub-group IC) genotypes. Group II consists of seven African genotypes only. GE 4927 from Zambia is present as an outlier. Most of these genotypes were clustered by geography. For example, the Indian and African genotypes were grouped in separate clusters, which may be due to the presence of distinct morphological characters between two sub-populations. Intermixing of African genotypes in Indian cluster was also observed, which suggested that Indian germplasm appears to be derived from African introductions. This also supports the previous report (Dida *et al.*, 2008; Arya *et al.*, 2013) that finger millet was first domesticated in Africa and then spread to India.

The results of the STRUCTURE analysis (Fig. 2) based on maximum Ln P(D) and peak value of delta K inferred the number of populations, K to be 2. Of the 40 finger millet genotypes, 34 (85.0%) shared > 70% membership with one of two sub-groups (G1 and

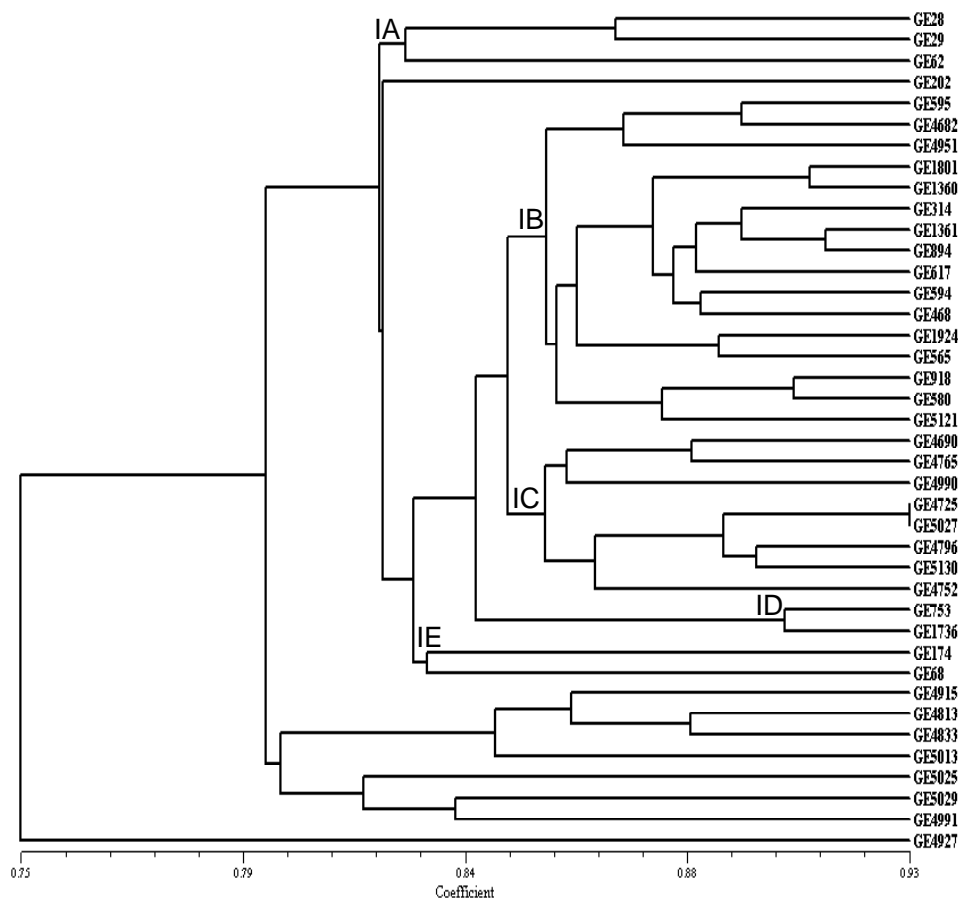
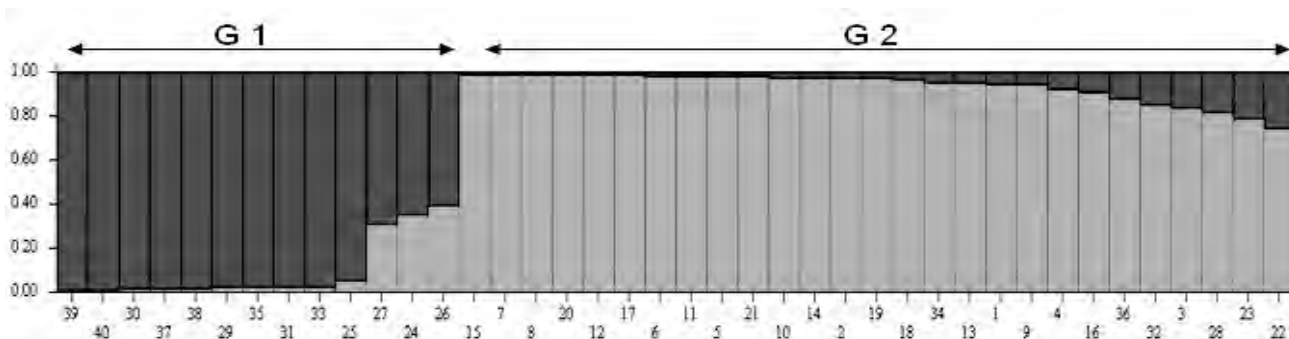


Fig. 1. UPGMA dendrogram based on RAPD marker data of 40 finger millet genotypes



**Fig. 2.** Population structure analysis of 40 finger millet genotypes generated by STRUCTURE 2.3.2 using the admixture model with correlated allele frequencies. Numbers on the y-axis show the subgroup membership, and the numbers on the x-axis show the genotype number. Different populations are represented by different colors at  $K=2$ , where  $K$  is the number of subgroups/subpopulations. Genotype numbers are: 1=GE28, 2= GE29, 3=GE62, 4=GE202, 5=GE595, 6=GE753, 7=GE1736, 8=GE1801, 9=GE174, 10=GE314, 11=GE617, 12=GE1361, 13=GE918, 14=GE580, 15=GE1360, 16=GE68, 17=GE894, 18=GE1924, 19=GE565, 20=GE594, 21=GE468, 22=GE4690, 23=GE4682, 24=GE4725, 25=GE4752, 26=GE4765, 27=GE4796, 28=GE5121, 29= GE5130, 30=GE4915, 31=GE4927, 32=GE4951, 33=GE5025, 34=GE5027, 35=GE5029, 36=GE4990, 37=GE4991, 38=GE4813, 39=GE4833, 40=GE5013

G2) and were classified as members of that sub-group (Table 1), whereas six genotypes (15.0%) were categorized as admixture forms with varying levels of membership shared between the two sub-groups. The mixture is likely the result of breeding (targeted or accidental intercrossing) and domestication history (Africa and India being the primary and secondary center of diversity for finger millet respectively), which have had large effects on the diversity structure. The independent population histories of the groups have shaped gene pools (Garris *et al.*, 2005). Sub-groups G1 included 13 (32.5%) genotypes (African genotypes), one each from Tanzania and Ethiopia, two each from Malawi, Zambia and Zimbabwe, and five from Kenya. Sub-group G1 included genotypes from all the African countries except Uganda which were grouped with Indian genotypes. This may be due to the presence of distinct morphological traits in Ugandan genotypes as is evident from our phenotypic data and the distinctness of Ugandan lines was also reported by earlier studies (Arya *et al.*, 2013). Whereas, sub-group G2 (dominant in Indian genotypes) included the remaining 27 (67.5%) genotypes, with 21 from India (one each from Karnataka, Kerala and Bihar, two from Madhya Pradesh, three from Uttaranchal, five from Andhra Pradesh and eight from Tamil Nadu), six from Africa (admixtures) [one each from Kenya (GE 5121), Zambia (GE 4951), Zimbabwe (GE 5027) and Tanzania (GE 4990) and two from Uganda (GE 4690, GE 4682)]. Same six accessions were reported as admixtures based on SSR based population structure analysis (Arya

*et al.*, 2013). This structuring is correlated well with primary and secondary centers of finger millet diversity and the two sub-populations (G1 and G2) basically form two distinct groups and can be used to augment finger millet in both India and Africa.

RAPD marker analysis very well revealed the geographic distribution pattern of genetic variation and population structure in the Indian and African finger millet and also supported the previous reports based on other marker systems emphasizing the role of RAPDs as effective markers for evaluating genetic diversity in finger millet. Our results indicate that Tamil Nadu followed by Andhra Pradesh region of India and in Africa, Zambia followed by Kenya are the diversity rich regions of finger millet. More number of finger millet germplasm should be collected from these places to capture the existing genetic diversity. On the other hand, in other finger millet growing regions, the germplasm may be collected from wider areas to collect existing variability. In summary, the results derived from genetic diversity and population structure analyses could be used for making efficient strategies for finger millet germplasm collection, conservation and designing effective breeding programs to broaden its genetic base.

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