Evaluation of Morphological and Molecular Diversity among the Genotypes of *Eleusine africana*, Cultivated and Weedy Types of Finger Millet (*Eleusine coracana* L. Gaertn.)

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A B S T R A C T

The present study was carried out to understand the extent of morphological and molecular variability between cultivated finger millet genotypes of Indian and African origin and weedy *E. coracana* and *E. africana* genotypes. A total of 28 genotypes representing cultivated varieties (*E. coracana*) from India and Africa, wild species (*E. africana)* and weedy types of *E. coracana* were grown in field and scored for 23 morphological traits. The African cultivated types were distinct from Indian types for many morphological traits. The *E. africana* and weedy *E. indica* genotypes formed a separate cluster based on qualitative traits as well as molecular markers. The clustering and multivariate analysis conducted with morphological as well as molecular data, could divide the cultivated genotypes separately from wild and weedy types. The weedy type EC516243 S1 having relatively bolder seeds and superior productivity related traits were identified as suitable breeding materials for hybridisation with cultivated types.

Introduction

Finger millet (*Eleusine coracana* L. Gaertn.) is a highly self-pollinated, allotetraploid (2n = 4x = 36) annual crop, member of the family Poaceae, sub-family Chloridiodeae and tribe Chlorideae and native to Uganda and Ethiopian regions of Africa (Harlan, 1971; deWet, 1995). In world, finger millet ranks fourth in importance among millets after sorghum, pearl millet and foxtail millet with major producers being Uganda, India, Nepal and China having a yield potential of more than 10 tonnes per hectares under irrigated conditions (Goron and Raizada, 2015). In

India, finger millet commonly known as ragi is cultivated in an area of 1190 thousand hectares with the average productivity of 1631 kg per hectares (AICRPSM, 2016). The production area of finger millet in India stands sixth after wheat, rice, maize, sorghum and bajra and Karnataka is the leading producer accounting to 58 per cent of its global production. Recently the area under finger millet is drastically reduced in all the major growing states of India, particularly Karnataka (Michaelraj and Shanmugam, 2013). However with global climate change and hidden hunger of the growing population, finger millet has a significant role to play in the future.

Therefore, there is a need to enhance the variability in the cultivated tetraploids with increased adaptability to climate change. In this direction, the tetraploid wild and weedy *Eleusine* species have a significant allelic contribution for adaptive agriculture.

The morphological and molecular diversity present among the different species of *Eleusine* is immense. Diffusion of the finger millet crop from its primary centre of diversity, situated in the East African regions where primitive cultivars and weedy forms exist (Chennaveeraiah and Hiremath, 1974) to other areas has led to the accumulation of diversity in secondary centre of diversity which is considered to be India (Dida *et al.,* 2008). However, the assessment of genomic relationships between cultivated *E. coracanca* and its wild tetraploid species has been limited. Assessment of the genetic diversity and relationships using microsatellites or SSR markers provide wide and unique opportunities to quickly and accurately access the allelic diversity variability between cultivated *Eleusine* species and other tetraploid types of *Eleusine* for their utilization in crop improvement programmes of the finger millet.

Materials and Methods

Plant material

The experimental plant material for the present investigation consisted of eleven cultivated genotypes of *Eleusine coracana* from India, six cultivated genotypes from Africa, nine *Eleusine africana* accessions and two weedy *E. coracana* from India (Table 1). The seeds of these genotypes were obtained from All India Coordinated Research Project on Small Millets (University of Agricultural Sciences GKVK), Bangalore. All the genotypes were germinated in the laboratory by providing seed treatment (0.3 % Hydrogen peroxide; Katzman *et al.,* 2001) and transplanted to field during kharif 2016 at UAS GKVK, Bangalore. Each genotype was transplanted in a single row of 2.5 meters length with 30 X 10 cm spacing. The crop was grown under irrigation with recommended agronomic practices.

Morphological characterization

For each genotypes five plants were randomly selected for recording observations on qualitative traits like growth habit, plant pigmentation at leaf juncture, leaf sheath pubescence, glume color, culm branching, ear shape, finger branching, position of finger branching, multiple whorl, seed shattering, seed covering by glumes, seed color, seed shape, seed surface as well as quantitative traits, including days to 50 % flowering, flag leaf blade length (cm), flag leaf blade width (cm), ear head length (cm), finger length (cm), finger width (cm), finger number, number of productive tillers /plant and plant height at maturity (cm) were recorded at different developmental stages as per the finger millet descriptor (IBPGR, 1985). The mean values were used for further analysis.

Isolation of genomic DNA

Fourteen genotypes (five cultivated genotypes of India, four cultivated genotypes of Africa, three *E. africana* accessions and two weedy types of *E. coracana*) were selected for molecular analysis. The genomic DNA was extracted from young leaves of single representative plant in each genotype following CTAB extraction method (mini preparation) as described by Doyle and Doyle (1987).

The DNA was diluted to 50 ng/ µl concentration for SSR genotyping after purity assessment in Nanodrop spectrophotometer (ThermoScientific, USA).

Microsatellite markers and PCR analysis

A set of 21 finger millet EST- SSR primer pairs (Table 2) were used for PCR amplification and molecular diversity analysis. The PCR amplification reactions were carried out in Mastercycler nexus gradient (Eppendorf, Germany) with 10 µl reaction mixture containing 50 ng of genomic DNA, 2mM dNTPs, 10 pMol each of forward and reverse primers, 1 U/µl *Taq* DNA Polymerase and 1X PCR buffer. The PCR was performed with an initial denaturation at 95˚C for 5 min followed by 38 cycles of 30 sec denaturation at 94˚C, 45 sec annealing at different temperatures based on primer pairs and 1 min extension at 72˚C with a final extension at 72˚C for 8 min. The PCR products were separated and visualized on a 3.5 % agarose gel and the fragment sizes were estimated by comparison with 100 base pair (bp) DNA ladder.

Statistical analysis

The hierarchical cluster of the genotypes was obtained separately for total morphological traits and qualitative traits by UPGMA (Unweighted Pair-Group Method using Arithmetic Average) analysis with Continuous (Usual Euclidean) dissimilarity index using DARwin 6 (Perrier X and Jacquemoud-Collet J. P. 2006) software. The qualitative characters that depict an array of characters were converted into binary characters based on various pretext in each trait. The quantitative data gathered on different traits were standardized to zero mean and a unit variance.

The amplicons were scored according to their product size for each genotype and primer combination. The allelic data were subjected to estimation of genetic distances among the genotypes. Each amplified band was treated as an independent locus and were scored based

on present (1) or absent (0) system. The Sequential Agglomerative Hierarchical Non overlapping (SAHN) clustering on squared Elucidean distance matrix and similarity matrix utilizing UPGMA method based on Jaccard similarity coefficient was employed for cluster analysis and estimation of genetic similarity respectively using the software package NTSYSpc 2.02i (Rohlf, 1998).

The estimation of the major allele frequency, allele number, gene diversity index and polymorphic information content (PIC) value was done using Power Marker v3.0 software (Liu and Muse, 2005). The principal component analysis (PCA) with data on nine quantitative morphological traits and principal coordinate analysis (PCoA) based on Jaccard similarity index with 21 EST-SSR marker scores were conducted separately using PAST v3.15 software (Hammer *et al.,* 2001).

Results and Discussion

Diversity analysis and grouping based on morphological data

The cultivated genotypes from Africa had differentiating characters from Indian cultivated types in terms of having dark green glume colour, presence of finger branching in all fingers and higher mean for quantitative traits like flag leaf blade length, ear head length, finger numbers on main ear and plant height at maturity (Table 3). The morphological differences between Indian and African cultivated accessions with respect to inflorescence characters have also been reported by Naik *et al.,* (1993), Upadhyaya *et al.,* (2006) and Babu *et al.,* (2014a). However, when compared among all four groups of genotypes, wild species (*E. africana*) and weedy types of *E. coracana* had higher mean for most of the quantitative traits. Therefore, the wild and weedy species can be exploited for improvement of quantitative traits in finger

millet (Mariam *et al.,* 1996). The EC516243 S1, a weedy type which had relatively bolder seeds, higher mean for finger length, ear head length, finger width and plant height was identified suitable for hybridization programmes with cultivated genotypes. Attempts are being made to cross EC 516243S1 to cultivated high yielding genotype (GPU 28) for introgression of the desirable traits.

The dendrogram based on UPGMA clustering constructed with 23 morphological parameters for 28 genotypes divided the genotypes into two major clusters (Fig. 1). The cluster I had 27 genotypes whereas the cluster II had only one accession, EC516243 S1 which was morphological distinct among all the genotypes. The first cluster was divided into several sub-clusters, however the grouping of genotypes didn't follow any specific pattern. To have a more credible picture of the morphological differences among the genotypes another dengrogram based on 14 qualitative traits was constructed which divided the genotypes into two major clusters (Fig. 2). The qualitative characters provide a means of characterizing and differentiating genotypes morphologically from one another. The cluster I had all the 17 cultivated genotypes (*E. coracana*) whereas all the 11 genotypes of wild species (*E. africana*) and weedy types were grouped in the cluster II. However, the clustering of cultivated genotypes was not based on geographical origins which is in accordance with the previous reports by Upadhyaya *et al.,* (2006) and Kumar *et al.,* (2010).

The principal component analysis (PCA) of 28 genotypes using nine quantitative traits revealed that the traits like ear head length, finger length, productive tillers / plant, plant height at maturity, finger width and flag leaf length contributed more to the diversity which is in agreement with the previous reports by

Reddy *et al.,* (2009), Nethra *et al.,* (2014), Ulaganathan and Nirmalakumari (2015) and Mahanthesha *et al.,* (2017). The highest contribution to the first principal component which accounted for 38.33% to the total variation came from finger length (0.47) followed by ear head length (0.46) (Table 4). The traits like finger numbers/ear head, finger width, flag leaf length and flag leaf width showed negative contribution. Similarly, the plant height contributed highest (0.52) followed by flag leaf length (0.43), to the second principal component which accounted for 23.27% to the total variation (Table 4). The characters like days to 50% flowering, productive tillers/plant and finger width contributed negatively. All the cultivated genotypes except GE 12 concentrated separately from genotypes of *E. africana* and weedy types in quadrant 2 and 3 of scatter bi-plot (Fig.3). GE 12, an Indian cultivated genotype was found to be distant from the cluster of other cultivated genotypes due to its distinct ear head shape of open type, highest ear head length, finger length and plant height among cultivated genotypes.EC516243 S1 (weedy type) and GE 7130 (*E. africana*) were dispersed distantly in PC1 and PC2 respectively which is in concordant with the pattern found in clustering analysis (Fig. 1& 2).

Diversity analysis and grouping based on molecular marker data

The present study showed relatively lower polymorphism (42.86%) with only nine out of 21 SSR markers were polymorphic among cultivated and other weedy genotypes. Babu *et al.,* (2014a, b) also reported low polymorphism in their study involving 190 cultivated genotypes and 95 SSR markers. The mean major allele frequency was higher for cultivated genotypes (0.94) as compared to the wild species (*E. africana*) and weedy types (0.89).

Table.1 Details of the plant material used in the present study

Sl. No.	Genotype	Country	Species
1.	GE 6	India	*E. coracana*
2.	GE 12		
3.	GE 13		
4.	GE 14		
5.	GE 1598		
6.	GE 1599		
7.	GE 1600		
8.	GE 1606		
9.	GE 1607		
10.	GE 2403		
11.	PR 202		
12.	GE 1595	Africa	
13.	GE 1612	Uganda	
14.	GE 1613		
15.	GE 1615		
16.	GE 1616		
17.	GE 1618		
18.	GE 7123	Not known	*E. africana*
19.	GE 7124		
20.	GE 7125		
21.	GE 7126		
22.	GE 7127		
23.	GE 7128		
24.	GE 7129		
25.	GE 7130		
26.	GE 7131		
27.	EC516243 IPS-3	Not known	Weedy type of *E. coracana*
28.	EC516243 S1		

Table.2 The sequence details of EST-SSR primers used for molecular diversity study and the number of alleles generated

Sl. No.	Primer	Sequence (5'-3')	Number of alleles
1.	FM 70	**F**- GAGTCTATGATCCGCTCTTG	1
		R- CACGAATTAGACATGGGATT	
2.	FM 77	**F**- GGGAGGTCGACTAAGCTG	2
		R- AACTTGTCCTGCATCATCTC	
3.	UGEP 26	**F**- ATGGGGTTAGGGTTCGAGTC	1
		R- TGTCCCTCACTCGTCTCCTC	
4.	UGEP 76	**F**- GCACGTACGGATTCACATTG	2
		R- GGTACGGAGACATCGACACC	
5.	UGEP 21	**F**- CAATTGATGTCATTGGGACAAC	2
		R- GTATCCACCTGCATGCCAAC	
6.	UGEP 110	**F**- AAATTCGCATCCTTGCTGAC	1
		R- TGACAAGAGCACACCGACTC	
7.	UGEP 77	**F**- TTCGCGCGAAATATAGGC	2
		R- CTCGTAAGCACCCACCTTTC	
8.	FMBLESTSSR 20	**F**- AGAGGAACCGCTCATTCAGATA	2
		R- GGGGTAGAAAGTCTGGGAAATC	
9.	FMBLESTSSR 13	**F**- GCTAAAGACAAAGAGTGCCCAT	2
		R- AAATATCCCTCTGCCACTTCCT	
10.	FMBLESTSSR 19	**F**- CCAACTTCTGGTTTTAACGTCC	1
		R- GTGGTAACTTTTCTGGCACCTC	
11.	FMBLESTSSR 43	**F**- GAGGCCCACACACTTCTTAGCGA	1
		R- GGAGTATGGGTAGTGGAACCAGTG	
12.	FMESTSSR 17	**F**- CATCTCCATCTCCATCTCCA	2
		R- AAGGACGATCGCAACCAG	
13.	FMESTSSR 21	**F**- CGCTAGTAGTACATCACAGCTA	1
		R- CTGATGGCGTATGGGAGTCT	
14.	FMESTSSR 16	**F**- GAGGCATGCACGTACAACAC	1
		R- GGAGGGAGGGAATTCACAAT	
15.	FMESTSSR 29	**F**- CCACCTGCTCCATCTACATCT	2
		R- AAGGACGATCGCAACCAG	
16.	FMBLESTSSR 3	**F**- GGATGAAGTGAGATTCCCCATA	2
		R- CAAGCTCTTCTGTGTTGCACTC	
17.	FMBLESTSSR 36'	**F**- CTCGCAGTGACAGGGCTT	1
		R- GTGTTTGTGTTGTGTGCTGTGT	
18.	FMBLESTSSR 2	**F**- TATGGGACATCTGCTCAAAGA	1
		R- AGAAGGCAGGTGAACGTAACTC	
19.	FMBLESTSSR 4	**F**- TTGTCTGGGATGACTTGAACAG	1
		R- AGCTCGTGGTACTTATGGAAGG	
20.	FMBLESTSSR 29	**F**- AGCTTTCGGAGGGAAGTTAGAC	1
		R- ACGGTTATGAGAAGTGGAAATG	
21.	FM SSR 01	**F**- GCGAAAACACAATGCAAAAA	1
		R- GCGTTGGTTGGACCTGAC	

Table.3 The mean±SE values for 9 quantitative morphological traits among the genotypes

| Sl. No.	Genotypes	N	Days to 50 % flowering	Plant Height (cm)	Productive tillers /plant	Finger number on main ear	Finger Width (cm)	Finger length (cm)	Ear head length (cm)	Flag leaf length (cm)	Flag leaf width (cm)
1	Cultivated genotypes from India	11	42.91 ±1.80	112.71±5.76	6.20 ±1.27	8.33 ±0.57	1.10 ±0.06	7.10 ±0.75	8.11 ±0.84	44.52 ±1.91	1.21 ±0.07
2	Cultivated genotypes from Africa	6	40.67 ±0.67	128.10±4.58	3.20 ±0.23	9.13 ±0.79	1.05 ±0.03	6.59 ±0.60	8.26 ±0.62	53.14 ±2.59	1.30 ±0.13
3	*E. africana*	9	44.44 ±1.39	117.17±8.29	13.14 ±2.20	8.53 ±0.46	0.50 ±0.13	13.31± 1.30	14.54± 1.32	38.31 ±3.72	0.99 ±0.07
4	Weedy types	2	53.00 ±3.00	160.22±20.04	12.40 ±4.60	9.40 ±0.00	0.53 ±0.05	18.60± 1.22	19.76± 1.42	33.93 ±13.35	1.19 ±0.21

(N= Number of genotypes)

Table.4 The contribution of nine quantitative morphological traits to the variation in PC1 and PC2

| Trait	PC1 (38.33%)*	PC2 (23.27%)*
A (Days to 50 % flowering)	0.30	-0.07
B (Plant height - cm)	0.23	0.52
C (Productive tillers / plant)	0.32	-0.45
D (Finger numbers/ear head)	-0.03	0.22
E (Finger width - cm)	-0.41	-0.04
F (Finger length - cm)	0.47	0.27
G (Ear head length - cm)	0.46	0.29
H (Flag leaf length - cm)	-0.31	0.43
I (Flag leaf width - cm)	-0.25	0.35

(*Values in parenthesis are Per cent variance explained by PC1 and PC2)

Fig.1 The dendrogram showing relatedness among 28 genotypes using 23 morphological traits
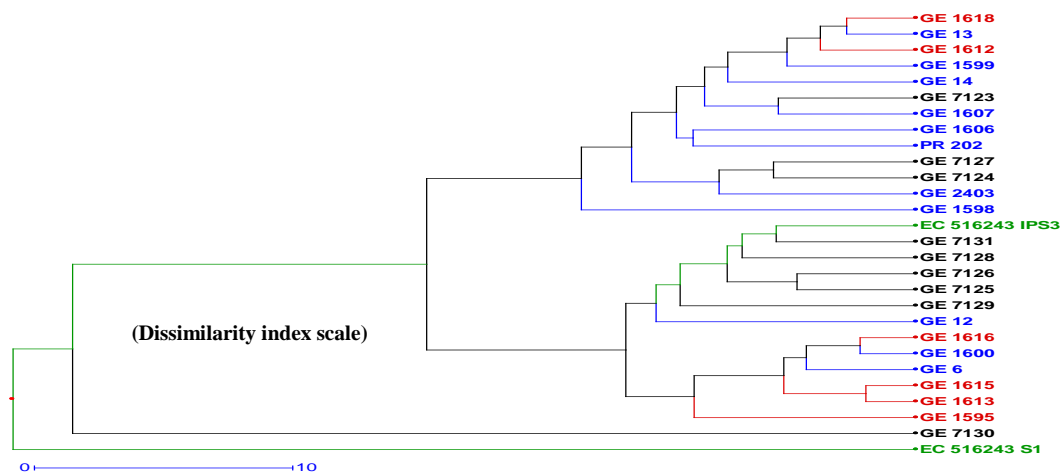
(**Blue**- Cultivated genotypes from India, **Red**- Cultivated genotypes from Africa, **Black**- *E. africana*, **Green**- Weedy types of *E. coracana*)

Fig.2 The dendrogram showing relatedness among 28 genotypes using 14 qualitative morphological traits
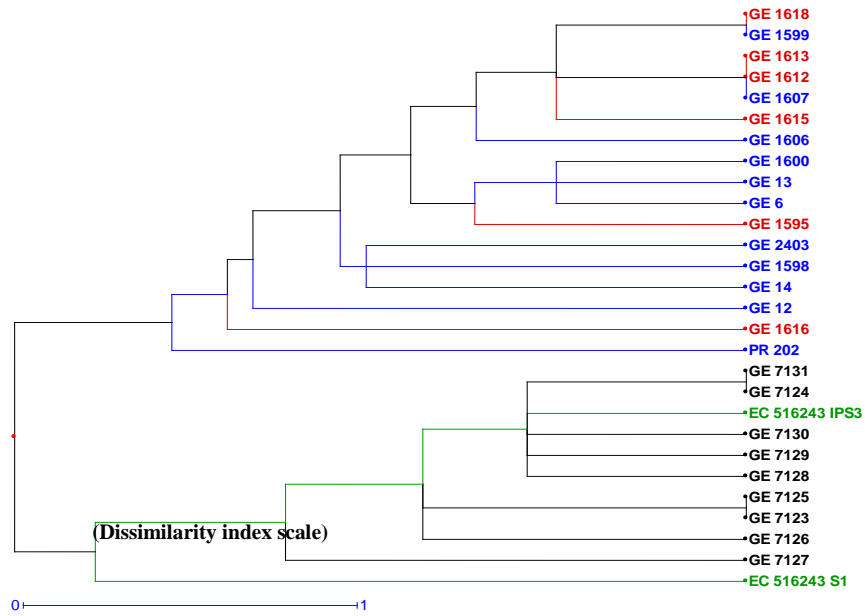
(**Blue**- Cultivated genotypes from India, **Red**- Cultivated genotypes from Africa, **Black**- *E. africana*, **Green**- Weedy types of *E. coracana*)

Fig.3 The principal component analysis scatter bi-plot showing the distribution of 28 genotypes of using nine quantitative morphological traits
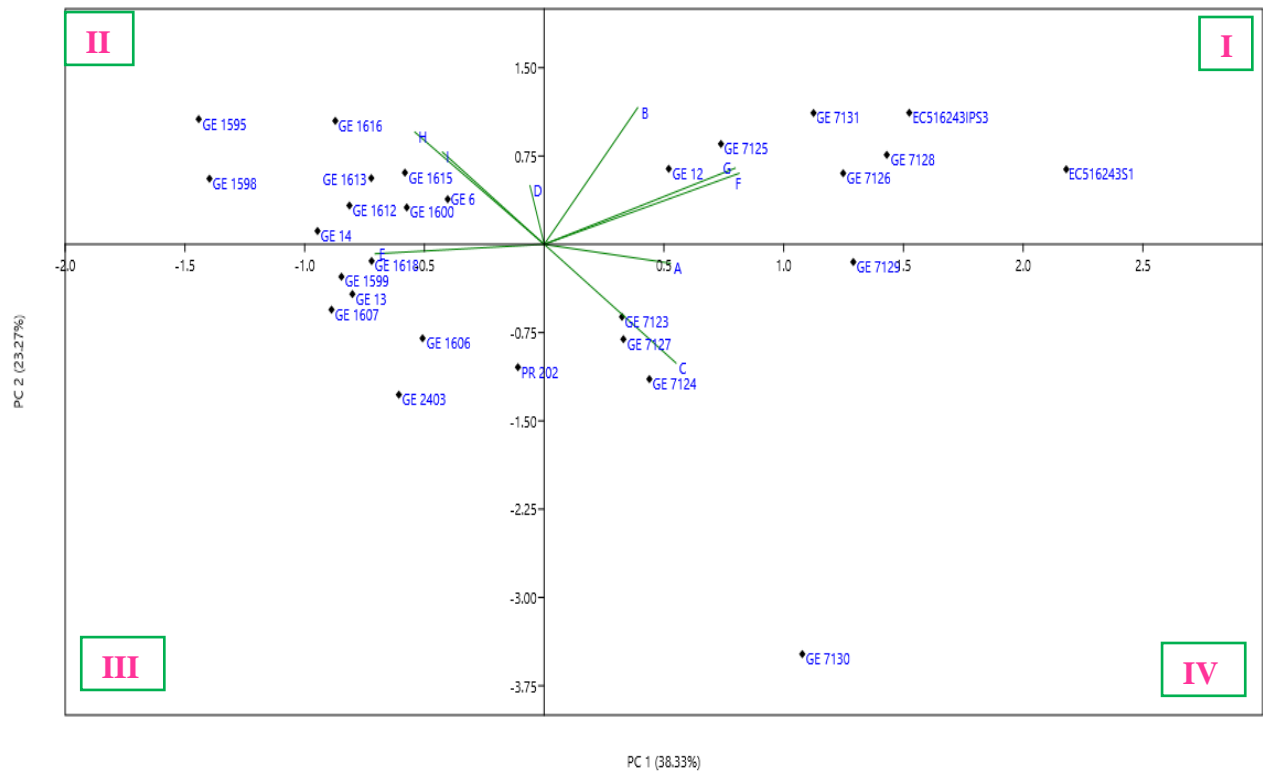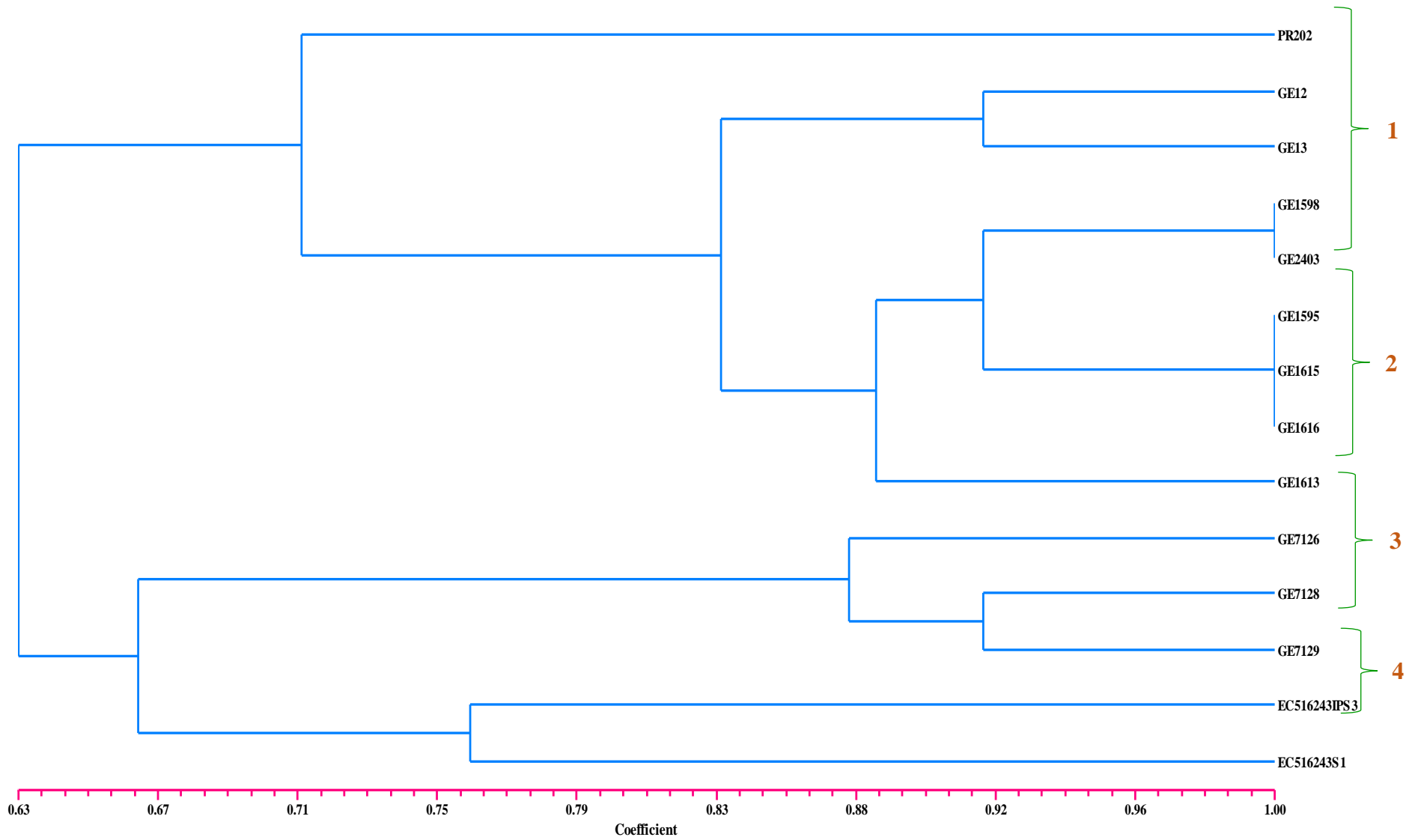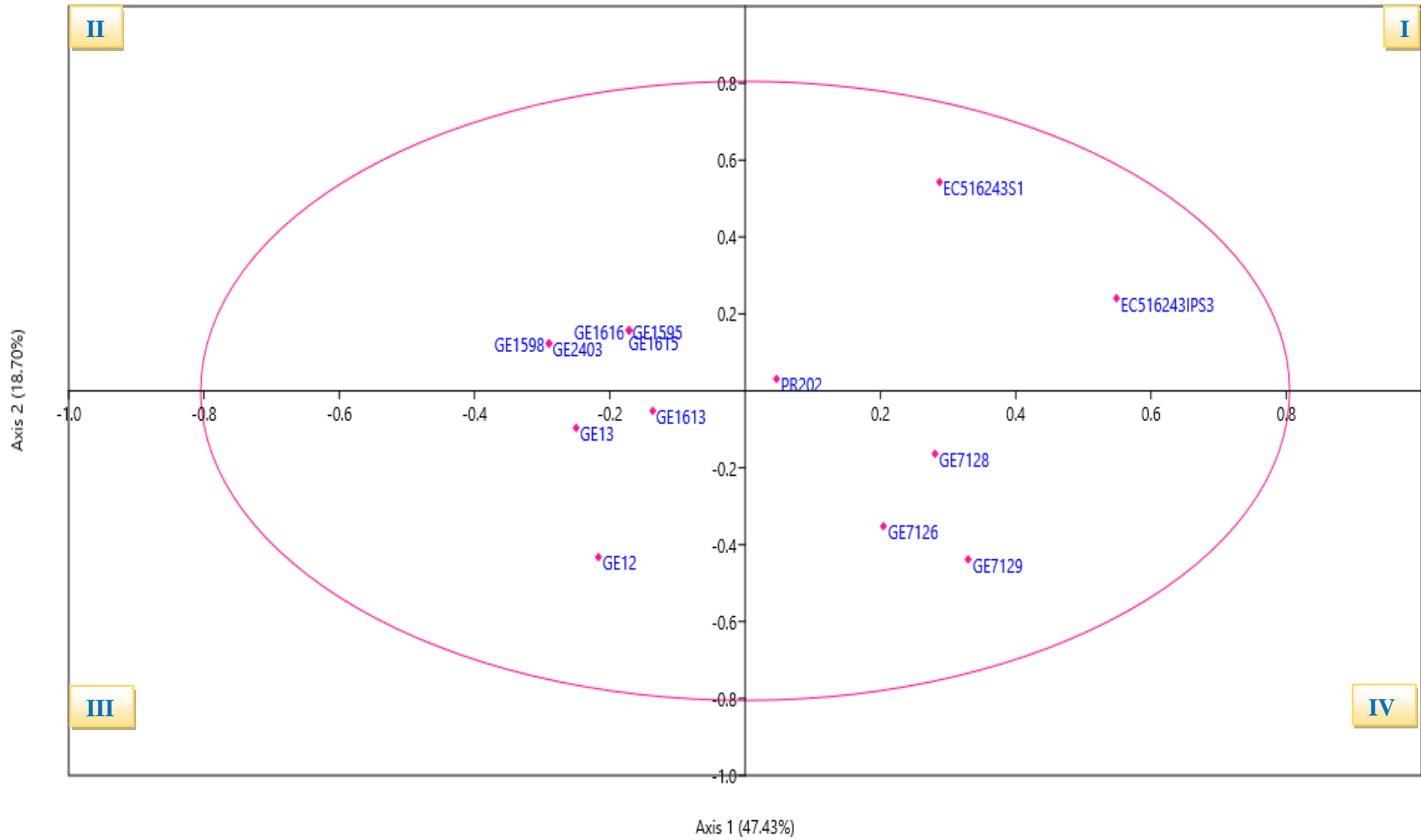
Fig.4 The dendrogram showing similarity relationship among 14 genotypes using SSR markers

(**1**- Cultivated genotypes from India, **2**- Cultivated genotypes from Africa, **3**- *E. africana,* **4**- Weedy types)

Fig.5 The principal coordinate analysis scatter plot showing the distribution of 14 genotypes using SSR markers

The polymorphic information content (PIC) for SSR loci ranged from 0.00 to 0.37, and the average PIC value was 0.13. The cultivated genotypes expressed lower mean genetic diversity index (0.08) and mean PIC value (0.06) as compared to the genetic diversity index (0.13) and PIC value (0.10) of wild species and weedy types. The lower genetic diversity in in cultivated finger millet genotypes has been reported by many workers earlier (Dida *et al.,* 2008; Arya *et al.,* 2013; Nethra *et al.,* 2014; Dagnachew *et al.,* 2014). The narrow genetic base among the cultivated genotypes could be due to domestication bottlenecks.

The cluster analysis based on UPGMA analysis divided the genotypes into two major clusters I and II (Fig. 4). All the nine cultivated genotypes (*Eleusine coracana*) were clustered in major cluster I while the genotypes of *E. africana* and weedy types were grouped in cluster II. Furthermore, genotypes of *E. africana* were clustered separately from weedy types in different sub-clusters of cluster II. But genotypes of cultivated types from India and Africa didn't segregate according to origin of collection. Such a mixed clustering of Indian and African cultivated genotypes has been previously observed by Arya *et al.,* (2013) and Ramakrishnan *et al.,* (2016).

The Principal Coordinate Analysis (PCoA) scatter plot demonstrated a comparable distribution of genotypesin different quadrants similar to the grouping revealed by cluster analysis. The contribution from first axis was 47.43% while the second axis contributed 18.70% to the total variation. The three cultivated genotypes of Africa *viz.* GE 1595, GE 1616 and GE 1615 were clumped at a point in the second quadrant.Both the weedy *E. coracana* types were grouped together in quadrant I along with cultivated genotype PR 202. (Fig. 5).

In summary, it can be observed that considerably lower genetic diversity was found in the cultivated types than in the wild samples. The cluster analysis and multivariate analysis conducted with morphological data was found to be consistent with that of analysis made with SSR marker data in separating the cultivated genotypes from wild and weedy types. The tetraploid weedy types having superior yield attributing traits could be an important source to enhance the diversity among the cultivated types through hybridization. Attempts are being made to cross the weedy species with cultivated genotypes to introgress the desired traits.

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