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RESEARCH ARTICLE

Morpho-molecular Variation in Indian Finger Millet (*Eleusine coracana* (L.) Gaertn.) Varieties and Landraces

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A total of 38 finger millet genotypes including 18 released varieties and 20 landraces from India were evaluated and compared based on RAPD, ISSR markers and morphological data collectively. RAPD markers showed higher polymorphism (61.62 %) as compared to ISSR (57.00 %) markers. The mean number of bands per primer and Jaccard's similarity coefficients generated for the markers RAPD, ISSR was 12.9; 0.810 and 14.3; 0.782 respectively. UPGMA clustering, and Structure analysis placed the landraces and varieties in separate groups with some exceptions. The data for 13 qualitative traits revealed diversity mainly for ear shape and ear size. ANOVA analysis of the 14 quantitative traits showed most of the characters to have significant difference, representing the presence of genotypic differences among the 38 genotypes studied. The morpho-variability as well as variation based on molecular markers was higher in the landraces compared to the varieties. The usage of more diverse landraces as genitors to augment the genetic base and identify genes for adaptive and nutritional variations as well as conservation of more landraces to maximize the available genetic diversity of finger millet was emphasized.

Key Words: ISSR, Landraces, Morphological, RAPD, Varieties

Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.), commonly known as ragi is an annual tetraploid and crop of immense importance due to its inherent resilience and nutritional qualities like high fibre, quality protein, rich mineral composition and neutraceuticals. It belongs to family Poaceae (Gramineae) and harbors enough genetic variability to be utilized for its further improvement as a nutritionally superior and subsistence crop for posterity. Finger millet is an important crop in India, particularly in the states viz. Karnataka, Tamil Nadu and Andhra Pradesh. Varieties used in the present study have been developed by pure line selection or hybridization (Indian × African; Indian × Indian). Indo-African crosses resulted in the development of many popular varieties. More and more finger millet genetic resources need to be characterized for their utilization as parents for development of improved varieties or generation of genomic resources. Landraces can serve as one such important genetically diverse plant genetic resource for crop improvement. Plant landraces were defined as "a dynamic population(s) of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems" (Villa *et al.*, 2005). Landraces were also defined as "balanced populations – variable, in equilibrium with both environment and pathogens and genetically dynamic" (Harlan, 1971). Landraces are the repertoire of diverse and rare alleles and are unique with respect to different traits of importance viz. tolerance to various biotic and abiotic stresses. They are sources of novel variation which must be characterized for their conservation, management and utilization.

Morpho-molecular characterization of the available genetic resources will help us in providing a holistic assessment of the level of existing genetic variation in finger millet. There are limited reports in which RAPD and ISSR markers were used for molecular characterization of finger millet (Fakrudin *et al*., 2004, Babu *et al*., 2007, Panwar *et al*., 2010; Ramakrishnan *et al*., 2015; Arya *et al*., 2016). There is always a scope for characterization of different set of germplasm using different markers in combination with its phenotypic

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characterization for effective utilization of crop genetic resources. In this study, released varieties and landraces (Karnataka, India) of finger millet were evaluated and compared using RAPD, ISSR markers and quantitative and qualitative traits collectively. The information generated through the morphological and molecular markers will complement each other in germplasm management including their utilization.

Materials and Methods

Plant Materials

For the current study, the seeds of 18 varieties were obtained from Regional Research Station, Mandya, Karnataka and 20 landraces were taken from the active collection at University of Agricultural Sciences (UAS), Bangalore (Table 1).

Table 1. Details of varieties and landraces of finger millet used for this study

S. No.	Variety/Landraces	Pedigreee / Source/place of collection	Year of release/collection
Varieties			
1	Poorna(IC075476)	CO 1 × Aruna	1959
2	Hamsa*(IC475470)	Selection from germplasm at Hebbal	1968
3	PR 202(IC402988)	Pure Line selection from dry ragi (Mettachodi) of Arakuvelly of Andhra Pradesh	1976 and 1982
4	INDAF 1(IC402565)	Pure line selection from African germplasm	1978
5	INDAF 3(IC402566)	Cauvery × IE 927	1978
6	Shakthi(IC75479)	Roo 13 × H 22	1978
7	INDAF 5(IC403042)	Cauvery × IE 927	1978
8	INDAF 7(IC402911)	Annapurna × IE 927	1984
9	INDAF 8(IC474181)	Hullubele × IE 929	1986
10	INDAF 9(IC403043)	K 1 × IE 980 R	1988
11	HR 911(IC403096)	UAS 1 × IE 927	1986
12	INDAF 15(IC403101)	IE 927 × IE 67	1991
13	MR 2(IC403094)	PR 202 × IE 927	1996
14	GPU 26(IC312307)	INDAF 5 × (INDAF 9 × IE 1012)	1998
15	MR 1(IC403093)	Hamsa × IE 927	1999
16	L5(IC312321)	Malavi × INDAF 9	1999
17	GPU 28(425957)	INDAF 5 × (INDAF 9 × IE 1012)	2000
18	GPU 45	GPU 26 × L 5	2001
Landraces			
19	GE 1296(IC475994)	Kari Ragi II, Karnataka	1977
20	GE 3353(IC478155)	Karimuddagaragi, Panchavalli, Karnataka	1981
21	GE 656(IC476023)	Kotekariaragi, Telaginakuppepura, PeriyapatnaTq. Karnataka	1977
22	GE3314(IC477427)	Devanagiriragi, Arabethittu, Herugur Taluka, Karnataka	1981
23	GE 741(IC476358)	Madaiahanagiriragi, Kolegala Taluk, Karnataka	1977
24	GE 3322(IC477483)	Doddaragi, Kolipalya, CR Nagar, Karnataka	1981
25	GE 3321(IC477614)	Karikaddiragi, Kolipalya, CR Nagar, Karnataka	1981
26	GE 1447(IC475267)	Beligiddaragi, Karnataka	1977
27	GE 1412(IC475467)	Hasiruragi, Karnataka	1977
28	GE 3371(IC477213)	Pullapudhugaragi, Gangadahosahalli, HD kote	1981
29	GE 1364(IC474936)	Shivpura local, Karnataka	1977
30	GE 736(IC475369)	Bettapura local, Periyapatna Taluka, Karnataka	1977
31	GE 658(IC476081)	Yadiayalla local, Gundlupet, Karnataka	1977
32	GE 1308(IC475800)	Hullupareragi, Karnataka	1977
33	GE 632(IC475694)	Handaragi, Hassan District	1977
34	GE 1343(IC475465)	Challekere local, Karnataka	1977
35	GE 702(IC475594)	Kari ragi I, Yaachathi, Gundlupet Taluka	1977
36	GE 776(IC475802)	Gangoor local III, Hassan Dist.	1977
37	GE 328(IC476251)	Mannurragi, Hassan Dist.	1977
38	GE 904(IC476104)	Hosakote local, Hosakoite, Karnataka	1977

*White seeded finger millet

Evaluation of Morphological Diversity

The selected finger millet genotypes were evaluated in Randomized Block Design (RBD) with number of replications (3), number of rows/plot (2), row length (2 m) and spacing (inter and intra row/22.5 × 15 cm) at UAS (University of Agricultural Sciences), Bangalore and ICRISAT, Hyderabad. Thirteen qualitative (plant pigmentation, ear shape, ear size, finger branching, discontinuity of spikelets on finger, synchrony of ear maturity, spikelet shattering, grain covering, grain colour, grain shape, grain surface, pericarp persistence, susceptibility to lodging) and 14 quantitative traits (plant height, days to flowering, days to maturity, culm thickness, productive tillers, leaf number, flag leaf length and width, peduncle length, finger number, finger length, finger width, test weight and grain yield), were recorded.

DNA Extraction and PCR Amplification

Extraction of total genomic DNA was carried out following cetyl-trimethyl-ammonium bromide (CTAB) method (Saghai-Maroof *et al*., 1984) with minor modifications using bulk leaves samples (30 individual plants/sample) of 6-week-old plant. DyNA Quant 200 fluorometer (Hoefer Instruments, USA) was used for quantification of DNA and a working concentration of 5 ng/µl was prepared for PCR.

RAPD Analysis

PCR amplification was carried out with 25 ng of genomic DNA, 3 mM $MgCl_2$ (Fermentas Life Sciences), 1U Taq DNA polymerase (Fermentas Life Sciences), 1x PCR buffer without $MgCl_2$ (Fermentas Life Sciences), 0.2 µM 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: 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.

ISSR Analysis

PCR amplification was carried out with 25 ng of genomic DNA, 2.5 mM $MgCl_2$, 1U *Taq* DNA polymerase, 1x PCR buffer without $MgCl_2$, 1.0 µM ISSR primer and 0.2 mM dNTP mix. Thermocycling conditions were as follows: denaturation at 94°C for 7 min.; thirty cycles of denaturation at 94°C for 30 sec., primer annealing at temperature specific to each primer for 45 sec. and primer extension at 72°C for 2 min. and final extension step at 72°C for 5 min.

Following PCR, RAPD and ISSR amplification products were loaded onto a 1.4% agarose gel in 1x TBE buffer and stained with EtBr. Electrophoresis was carried out at 90 V for 1.5 hours, followed by 70 V for 2 hours. The resolved RAPD products were visualized by UV and recorded using a Bio Imaging System (SynGene).

Statistical Analyses

Statistical analysis of morpho data was carried out using INDOSTAT package. Univariate analysis (ANOVA) and multivariate (cluster and ordination) analysis were performed for statistical analysis of quantitative traits and percentage frequency distribution was calculated for qualitative traits. RAPD and ISSR fragments were scored visually, as absent (0) or present (1). Jaccard's similarity coefficient was calculated for UPGMA clustering using NTSYS-pc. ver. 2.1 (Rohlf, 2000). GenAlEx software was used to calculate AMOVA (Peakall and Smouse, 2012). In addition, the software STRUCTURE (Pritchard *et al*., 2000) was used to investigate number of sub-groups using a burn-in of 100,000, a run length of 10,00,000 (admixture model). The number of sub-groups (K) in the population was determined by running the program at different K values (1 to 10) with five independent runs for each K value. Peak value of delta K was calculated using Structure Harvester (Earl and vonHoldt, 2012) to confer the number of distinct sub-groups.

Results and Discussion

Morphological Characterization

The data for 13 qualitative traits was recorded at Bangalore location and percentage frequency distribution was computed and cluster analysis was done using weighted average linkage dendrogram. Diversity was revealed mainly for ear shape and ear size. Semi-compact ear shape was found to be pre-dominant particularly among the varieties, while the landraces possessed both semi-compact and compact ear shapes. The varieties had a higher frequency of large ear size, but intermediate ear size was more pronounced in the material studied in general, and the landraces in particular. Semi-compact ear type and large ear of varieties may be due to the use of African germplasm as one of the parents in most of the varieties (Naik *et al*., 1993).

Analysis of variance (ANOVA) for 14 quantitative traits revealed significant differences for all the traits namely days to flowering, days to maturity, culm thickness, productive tillers, leaf number, flag leaf length and width, peduncle length, finger number, finger length, finger width, test weight and grain yield at both the locations. Combined ANOVA analysis showed most of the characters to have significant difference, indicating the presence of genotypic differences among the materials evaluated (Table 2). For location treatment all the characters showed significant difference. Number of productive tillers, flag leaf blade length and peduncle length were found to be non-significant for location and grain yield was observed to be non-significant for treatment. At both the locations, number of productive tillers showed a positively skewed distribution and high Kurtosis value (Table 3 and 4), indicating that the frequency distribution for this character is not strictly continuous. Differences were observed in the mean performance of the landraces and varieties between the two locations. The phenotypic coefficient of variation was high for characters such as, number of productive tillers, leaf number and finger length at both the locations. The variability was noticed to be higher in the landraces compared to varieties. In cluster analysis, the association between the clusters based on qualitative and quantitative characters was not distinct. But, some of the varieties, such as MR 1, MR 2, Indaf 15 and Indaf 8 grouped in one cluster; the varieties GPU 45, Indaf 5 and GPU 26 in a separate cluster and Hamsa and PR 202 grouped in a third cluster along with the landraces for both qualitative and quantitative characters. The inter-cluster distances for both locations were observed to be maximum between the cluster consisting predominantly of landraces and the cluster having mostly varieties. The landraces GE 632, GE 776, GE 328 collected from Hassan district and those collected from Kolipalaya namely GE 3321 and GE 3322 did not cluster together, indicating that the diversity pattern did not have strong association with geographical distribution.

Principal component analysis performed on standardized quantitative traits showed that first three most informative components accounted for 66.79% and 64.15% variation (Table 5), at Bangalore and Hyderabad location respectively. At Bangalore location important characters with greater weightage in principal component axis I were grain yield, days to maturity and days to flowering. Important characters in principal axis II

Table 2. Combined analysis of data for quantitative traits of locations, Bangalore and Hyderabad

Source	Df	DFL	PH	CT	PT	LN	FLBL	FLBW	PL	FN	FL	FW	DM	TW	GY
Location (L)	1	4522.98	21643.84**	9.05**	1.72^{ns}	346.84**	129.59**	0.02^{ns}	5.92^{ns}	4.43**	8.02*	0.86**	3533.71**	31.67**	510.63**
Replication (R)	4	5.93	609.28	0.026	1.45	6.82	50.55	0.06	17.19	0.16	0.86	0.04	39.57	0.19	11.83
Treatment (T)	37	234.28**	780.43**	0.05**	2.35**	29.49**	28.58*	0.07**	34.21**	3.46**	8.66**	0.02*	324.02**	0.46**	57.58^{ns}
L x T	37	14.73**	196.44**	0.006**	0.74**	8.84**	13.64**	0.14**	9.39**	0.58**	1.50**	0.01**	31.12**	0.20**	37.10**
Error	148	4.26	37.29	0.002	0.19	1.13	7.22	0.006	2.4	0.18	0.16	0.003	5.68	0.07	2.325

*P≤0.05, ** p ≤0.01, ^{ns}Not significant

Df= degree of freedom; DFL= Days to flowering; PH= Plant height; CT= Culm thickness; PT= Number of productive tillers; LN= leaf number on main tiller; FLBL= Flag leaf blade length; FLBW= Flag leaf blade width; PL= Peduncle length; FN= finger number; FL= Finger length; FW= Finger width; DM= Days to maturity; TW= Test weight; GY= Grain yield per plant

Table 3. Range of variation for quantitative traits at Bangalore location

Characters	Lowest	Highest	Mean	Kurtosis	Skewness	Standard Deviation	C.V
DFL	53.00	85.00	65.54	0.46	0.87	7.81	11.92
PH	46.85	119.40	81.06	-0.30	0.20	14.72	18.15
CT	0.73	1.19	0.93	-0.19	0.28	0.10	10.46
PT	2.33	7.30	3.80	3.41	1.58	0.92	24.32
LN	9.90	21.80	14.70	-0.43	0.27	2.46	16.73
FLBL	20.34	40.98	29.94	0.72	0.04	3.62	12.11
FLBW	0.76	1.32	1.05	-0.54	-0.17	0.12	11.76
PL	15.80	29.50	23.04	-0.48	-0.14	2.92	12.70
FN	4.80	10.10	7.14	0.83	0.32	0.92	12.83
FL	4.14	10.00	6.76	-0.34	0.06	1.20	17.83
FW	0.80	1.30	1.06	0.63	-0.30	0.09	8.16
DM	95.00	126.90	108.27	-0.32	0.51	8.93	8.25
TW	1.25	3.19	2.35	0.56	-0.26	0.36	15.32
GY	6.35	22.40	14.11	-0.73	-0.04	3.93	27.84

Df= degree of freedom; DFL= Days to flowering; PH= Plant height; CT= Culm thickness; PT= Number of productive tillers; LN= leaf number on main tiller; FLBL= Flag leaf blade length; FLBW= Flag leaf blade width; PL= Peduncle length; FN= finger number; FL= Finger length; FW= Finger width; DM= Days to maturity; TW= Test weight; GY= Grain yield per plant

Table 4. Range of variation for quantitative traits at Hyderabad location

Characters	Lowest	Highest	Mean	Kurtosis	Skewness	Standard Deviation	C.V
DFL	60.00	91.00	74.28	0.55	0.41	6.63	8.92
PH	68.40	127.10	100.37	-0.06	-0.30	11.76	11.72
CT	1.12	1.60	1.33	-0.24	0.22	0.10	7.90
PT	2.50	5.80	3.62	1.52	0.90	0.65	18.10
LN	10.60	24.70	17.04	-0.08	0.59	2.83	16.62
FLBL	20.82	35.30	25.52	0.56	0.06	3.78	9.98
FLBW	0.69	1.41	1.07	0.16	-0.44	0.13	12.56
PL	15.17	29.92	22.80	-0.21	-0.16	2.91	12.76
FN	5.30	9.50	7.42	0.22	-0.19	0.84	11.39
FL	4.37	9.77	7.14	-0.96	-0.06	1.45	20.28
FW	1.00	1.35	1.18	-0.46	0.35	0.08	6.65
DM	102.00	135.00	117.04	0.01	0.33	7.65	6.53
TW	2.10	4.80	3.10	2.12	-0.61	0.42	13.69
GY	9.42	29.70	17.12	-0.31	-0.03	4.29	25.06

Df= degree of freedom; DFL= Days to flowering; PH= Plant height; CT= Culm thickness; PT= Number of productive tillers; LN= leaf number on main tiller; FLBL= Flag leaf blade length; FLBW= Flag leaf blade width; PL= Peduncle length; FN= finger number; FL= Finger length; FW= Finger width; DM= Days to maturity; TW= Test weight; GY= Grain yield per plant

included flag leaf blade width and number of productive tillers. Finger number and peduncle length were the characters with greater weightage in principal component axis III. At Hyderabad location, days to flowering, leaf number and finger length had more weightage in principal component axis I. Finger number and flag leaf blade width were important in principal axis II, and peduncle length and flag leaf blade length in axis III. In general principal component analysis confirmed the groupings of the accessions obtained through cluster analysis.

Molecular Characterization

A total of 271 RAPD bands were generated using 21 random primers in the size range (250 to 3000 bp) with 61.62% polymorphism (Table 6). The number of bands generated per primer ranged from 5 (OPA07) to 23 (OPB11) with a mean of 12.9 bands per primer, and is higher than the earlier reports (Fakrudin *et al*., 2004, Babu *et al*., 2007, Panwar *et al*., 2010), which may be due to the primers selected for this study. For RAPD primers landraces showed 53.61% polymorphism as compared to 47.26% in finger millet varieties (Fig. 1).

Seven ISSR primers generated 100 PCR products in the size range 150 to 2200 bp and showed 57% polymorphism (Table 6). Eleven [(GA)9T] to 18 [(GA)9AAA(GA)5] ISSR bands were generated with a mean of 14.3 bands per primer. Landraces showed

Table 5. Principal components analysis for quantitative traits at both locations

PC Axes	Total variation explained		Character Weightage
	Percent	Cumulative	
Bangalore			
I	35.27	35.27	Grain yield (0.80), days to maturity (0.74), days to flowering (0.71)
II	17.72	52.98	Flag leaf blade width (0.75), number of productive tillers (0.69)
III	13.80	66.79	Finger number (0.65), peduncle length (-0.51)
Hyderabad			
I	39.04	39.04	Days to flowering (0.81), leaf number (0.80), finger length (0.76)
II	14.60	53.64	Finger number (0.70), flag leaf blade width, (-0.70), culm thickness (-0.49)
III	10.50	64.15	Peduncle length (-0.65), flag leaf blade length (-0.40)

53% and varieties 46.47% polymorphism with 100 ISSR markers (Fig. 1). For both the markers combined 53.44% and 47.04% of bands were polymorphic for landraces and varieties respectively.

The genetic similarity was determined on the basis of Jaccard's similarity coefficients. The mean values of the Jaccard's similarity coefficients for the markers RAPD, ISSR and both combined were 0.810, 0.782 and 0.802 respectively. The mean values for the markers combined were 0.824 for the varieties and 0.807 for the landraces. Both the %polymorphism and Jaccard's similarity coefficients values indicated that landraces were more diverse than varieties of finger millet.

For cluster analysis (Fig. 2) combined data of both the marker systems was used and two distinct clusters were obtained. Sixteen varieties along with one landrace GE 1343 formed cluster II and eighteen landraces along with only white seeded variety Hamsa formed cluster I, while GE 328 and variety Indaf 3 were present as outliers. The results indicated that all the varieties bred using African germplasm were closely related to each other and grouped in sub-cluster IIB and the varieties which were developed as pure line selections from India or Indian x Indian crosses were placed in sub-cluster IIA or as outlier of cluster II. Further in sub-cluster IIB varieties having common parents or showing pedigree relationship were closely grouped viz. MR 1 and MR 2; L 5 and Indaf 9; GPU 26, GPU 28 and GPU 45. Regarding landraces which were grouped in cluster I, the landraces GE 632, GE 776, GE 328 collected from Hassan district were placed separately like morphological markers but GE 3321 and GE 3322 collected from Kolipalaya clustered together, indicating that the molecular marker based diversity pattern have some association with geographical distribution.

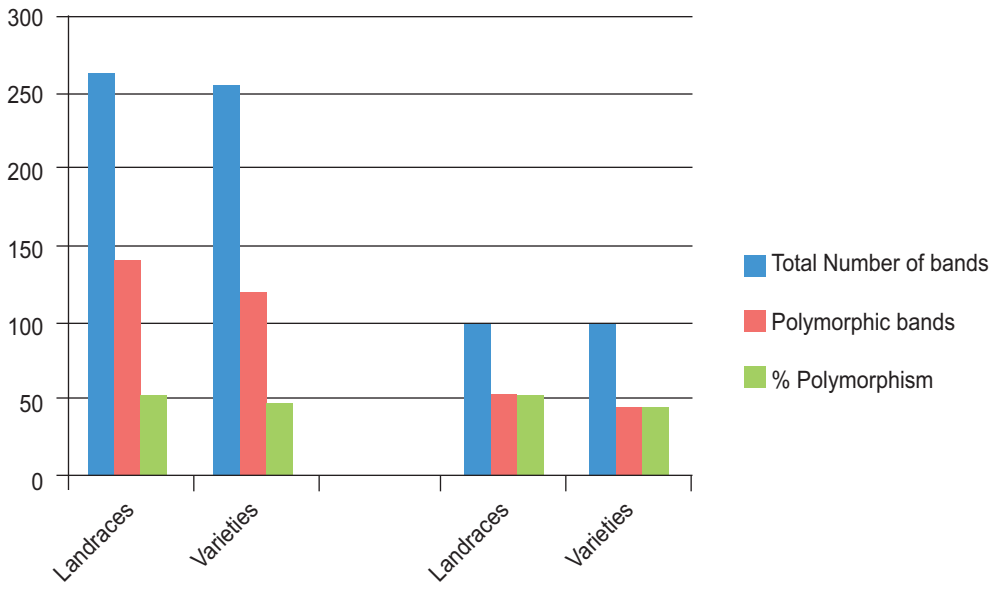

Fig. 1. Polymorphism summary statistics of landraces and varieties of finger millet based on RAPD and ISSR markers.

Table 6. Characteristics of bands generated using RAPD and ISSR primers

Primer	Total bands (no.)	Polymorphic bands (no.)	Polymorphism (%)	Size range of bands (bp)
RAPD Primers				
OPA 1	9	6	66.67	700-3000
OPA 7	5	4	80.00	500-2000
OPA 8	12	6	50.00	350-2500
OPA 13	17	15	88.24	300-2500
OPA 18	13	8	61.54	800-2000
OPB 4	14	8	57.14	500-3000
OPB 7	13	9	69.23	400-2000
OPB 8	13	7	53.84	550-3000
OPB 11	23	19	82.62	300-2400
OPB 12	7	5	71.43	450-900
OPB 18	14	11	78.57	300-2000
OPC 2	12	6	50.00	550-1800
OPC 6	10	4	40.00	600-2000
OPC14	19	11	57.89	450-3100
OPD 5	11	6	54.54	250-2000
OPD 8	16	7	43.75	750-2000
OPD 13	11	8	72.72	550-2000
OPK 1	11	6	54.54	400-2500
OPK 4	13	7	53.85	500-1900
OPF 14	14	6	42.86	400-2500
OPF 20	14	8	57.14	525-2300
Total	271	167	61.62	
ISSR Primers/Annealing temperature				
(GA)9T, 52°C	11	3	27.27	350-1400
(GA)9AC, 52°C	14	4	28.57	250-1500
(GA)9AY, 52°C	13	8	61.54	300-1400
(ACC)6T, 60°C	13	5	38.46	250-2000
(GACA)4, 52°C	14	10	71.43	300-2200
(AT)3(GT)15, 64°C	17	13	76.47	200-1800
(GA)9AAA(GA)5, 64°C	18	14	77.78	150-1500
Total	100	57	57.00	
Combined total	371	224	60.38	

Population structure analysis (Fig. 3) using RAPD and ISSR combined data resulted in to two groups, Group I (Varieties) and Group II (Landraces) based on peak value of delta K. All the landraces except GE 1447 and GE 1343 were in Group II and all the varieties except Hamsa, PR 202 and Indaf 3 were falling in Group I. All the varieties except Hamsa, PR 202, Indaf 3 and Poorna and all the landraces except GE 1447, GE 1412 and GE 1343 showed >86% membership coefficient in their respective groups (Table 7).

AMOVA was also conducted to analyze the separation between finger millet varieties and landraces. Between-variance component accounted for 14% variation compared to 86% within-variance component (Table 8). Results revealed that the level of genetic differentiation between the landraces and the varieties was low compared to variations within these groups. The probable reason may be that landraces might have been used in the development of these varieties as one of the parent along with African/Indian germplasm as the other parent in the lineage. Another reason for low component variance between the two groups could be that most of the landraces and varieties used in this study have originated/developed in Karnataka state of India. The landraces studied showed more diversity than varieties. The reason for this higher diversity may be due to the reason that landraces have been shaped over time due to natural selection by dynamic environmental conditions and human mediated selection. Forces like gene flow, selection, mutations and genetic drift

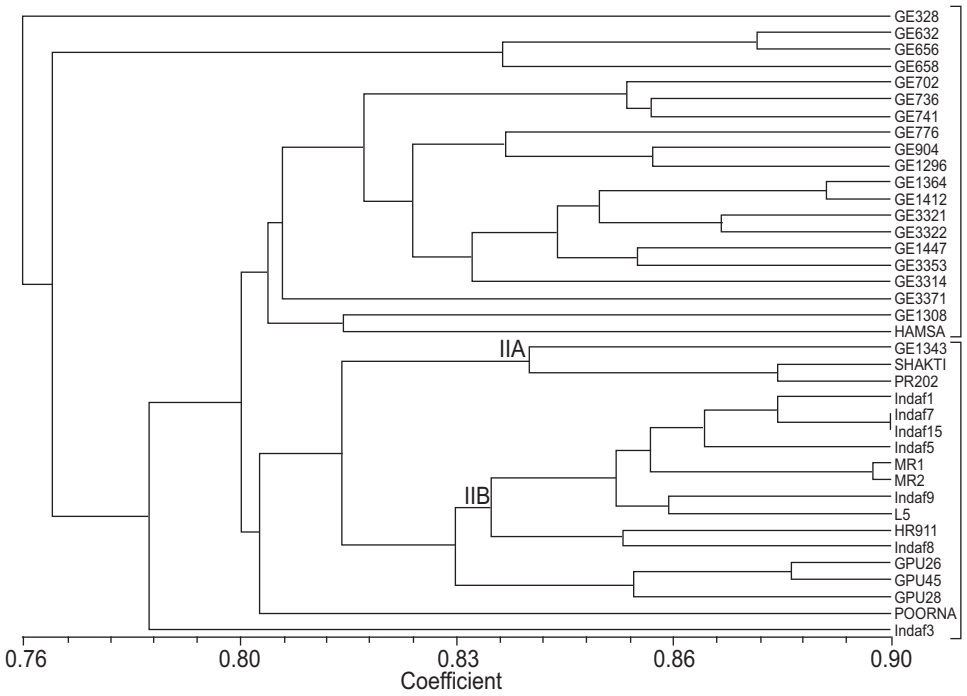

Fig. 2. UPGMA cluster analysis of finger millet varieties and landraces based on cumulative marker data (RAPD and ISSR).

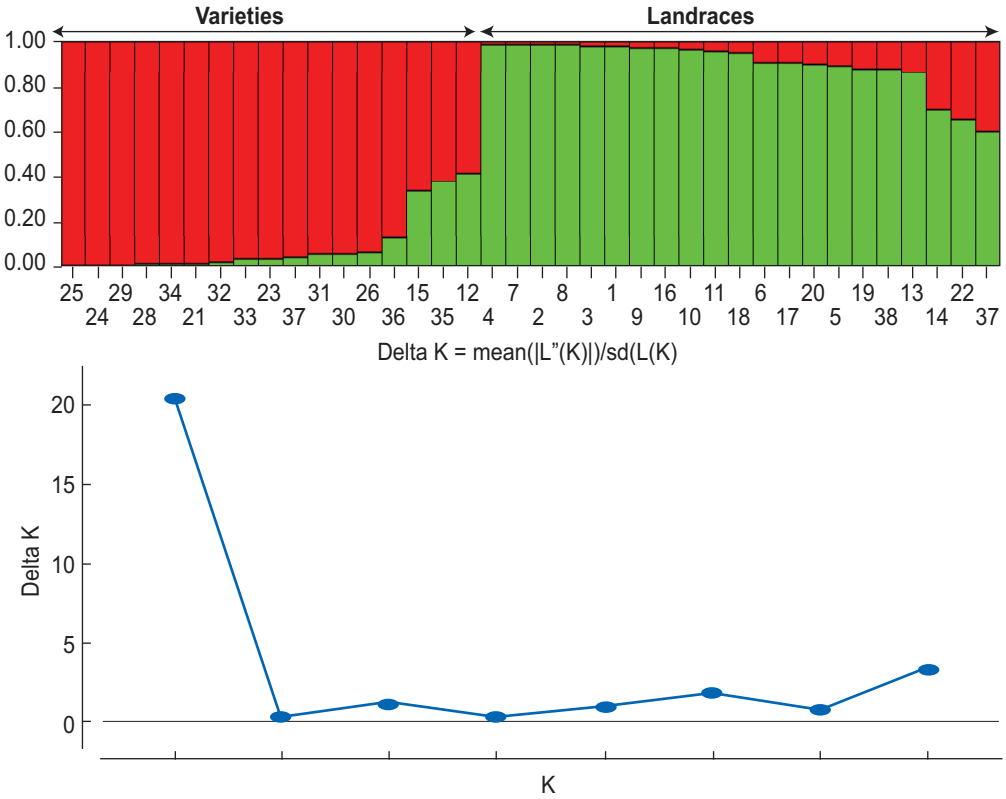

Fig. 3. Population structure analysis of 38 finger millet varieties and landraces and ΔK to predict the subgroups (Varieties and Landraces); 1: GE328, 2: GE632, 3: GE656, 4: GE658, 5: GE702, 6: GE736, 7: GE741, 8: GE776, 9: GE904, 10: GE1296, 11: GE1308, 12: GE1343, 13: GE1364, 14: GE1412, 15: GE1447, 16: GE3314, 17: GE3321, 18: GE3322, 19: GE3353, 20: GE3371, 21: Indaf1, 22: Indaf3, 23: Indaf5, 24: Indaf7, 25: Indaf15, 26: HR911, 27: Indaf8, 28: Indaf9, 29: L5, 30: GPU26, 31: GPU28, 32: GPU45, 33: MR1, 34: MR2, 35: POORNA, 36: SHAKTI, 37: PR202, 38: HAMSA.

Table 7. Membership coefficient of finger millet landraces and varieties in respective groups

Label	GI	GII
GE328	0.017	0.983
GE632	0.013	0.987
GE656	0.016	0.984
GE658	0.01	0.99
GE702	0.101	0.899
GE736	0.089	0.911
GE741	0.011	0.989
GE776	0.013	0.987
GE904	0.024	0.976
GE1296	0.031	0.969
GE1308	0.041	0.959
GE1343	0.582	0.418
GE1364	0.134	0.866
GE1412	0.297	0.703
GE1447	0.663	0.337
GE3314	0.024	0.976
GE3321	0.089	0.911
GE3322	0.049	0.951
GE3353	0.115	0.885
GE3371	0.096	0.904
Indaf1	0.981	0.019
Indaf3	0.337	0.663
Indaf5	0.962	0.038
Indaf7	0.99	0.01
Indaf15	0.991	0.009
HR911	0.93	0.07
Indaf8	0.951	0.049
Indaf9	0.984	0.016
L5	0.986	0.014
GPU26	0.936	0.064
GPU28	0.941	0.059
GPU45	0.973	0.027
MR1	0.964	0.036
MR2	0.983	0.017
POORNA	0.621	0.379
SHAKTI	0.865	0.135
PR202	0.397	0.603
HAMSA	0.12	0.88

make them more diverse. So there is a need to study landraces from different geographical areas in India at molecular and phenotypic level in order to explore their inherent genetic variability for utilization in crop improvement.

Comparison of molecular and morphological data revealed that the UPGMA clusters for RAPD and ISSR markers to some extent were similar to the clustering pattern based on quantitative characters. The landrace GE 1343 was found clustered with the varieties in quantitative trait analysis at Bangalore and dendrograms generated by RAPD and both the markers combined. The varieties Hamsa and PR 202, which were pureline selections from Indian germplasm were found to cluster with the landraces in Struture analysis based molecular and morphological characterization. Similarly, the varieties, such as MR 1, MR 2 and Indaf 15 grouped in one cluster and the varieties GPU 45 and GPU 26 in a separate cluster for qualitative, quantitative characters and molecular data.

Table 8. AMOVA of finger millet landraces and varieties based on ISSR and RAPD markers

Level of variation	df	SS	MS	Est. Var.	%
Among pops	1	114.388	114.388	4.514	14
Within pops	36	1039.033	28.862	28.862	86

degree of freedom (df); sum of squares (SS); mean of squares (MS)

In the present study, the materials evaluated showed significant genotypic variation. The morphological characterization accounted for higher variation than molecular characterization, which may be due to the reason that morphological traits are generally believed to be subject to natural selection and their expression is partly under the influence of environmental factors. Further, in contrast to morphological traits, molecular variation is based on DNA sequence variation. Molecular markers used are neutral and are not linked to any specific morphological adaptation, so the differences in diversity pattern were obvious.

The separate clustering of varieties in one group and the landraces in a separate group was more pronounced in the clustering patterns obtained through molecular markers. The forces causing high molecular differentiation could be due to genetic drift and no selection, particularly for landraces.

Conclusion

The landraces were found to be more diverse than the varieties and it is inevitable to introgress genes from landraces in to varieties to enhance the genetic base of finger millet and conserve more landrace accessions to maximize the diversity. Landraces can also serve as crucial genetic resources for association studies of genes responsible for adaptive variations, suggesting the *in situ* conservation of these landraces in the perspective of future climate change.

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