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Original Article

Genetic characterization of Indian little millet (*Panicum sumatrense*) genotypes using random amplified polymorphic DNA markers

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ABSTRACT

Initially, 60 primers were screened among 32 genotypes of little millet (*Panicum sumatrense*), from which 36 primers were selected on the basis of a sharp and clear banding pattern for final random amplified polymorphic DNA (RAPD) analysis. The PCR reaction was carried out using a single decamer primer at a time. In total, 175 RAPD marker loci were amplified of which 155 were polymorphic (88.58%). The band size of amplified markers was in the range 100–1900 bp. Ten bands were the maximum scored (for primer OPH-19) whereas two bands were the minimum scored (for primer OPC-13). On average, 4.86 bands per primer and 4.31 polymorphic bands per primer were recorded. The polymorphism information content ranged from 0.102 (OPAI-01) to 0.517 (OPE-04). A dendrogram divided the genotypes into two major and further into sub-groups according to their collection center geographical regions. This was the first report on molecular diversity analysis of a large collection of little millet germplasm. Diverse genotypes may be used in breeding programmes for *P. sumatrense* improvement.

Introduction

Tribal areas in India are known for their rich genetic diversity in various crops including millets. Little millet (*Panicum sumatrense*), belongs to the Poaceae family, is an annual erect and self-pollinating tetraploid (2n = 36) crop commonly known as 'Kutki'. It is widely grown in marginal areas and is associated with tribal agriculture being a predominantly rainfed crop with poor management by resource-poor farmers (Kumar et al., 2017). Sparse and irregular cultivation of little millet has led to less understanding of its genetic diversity (Selvi et al., 2015). In India, little millet is generally grown on light red soils and hillsides as a rainfed crop, which is usually never irrigated (Mall and Tripathi, 2016). Little millet is rich in vitamin B and has high nutritional value especially due to the presence of phosphorus and iron (Mall and Tripathi, 2016). As little millets contain no gluten, it has become a natural choice for those with celiac disease or other forms of allergies and intolerance of wheat (Saturni et al., 2010). It contains proteins (9.80–12.49 g/100 g), fat (2.87–5.09), ash (0.98–4.78), crude fiber (0.49–8.72) and carbohydrates (62.25–76.59 g/100 g) with various other important minerals (Usha et al., 2011).

Little millet is widely distributed in the temperate zone of Asia and tropical regions of the world (Baker, 2003). While genetic diversity is the base for crop genetic improvement and important for the conservation, evaluation, and utilization of crop germplasms, little millet is the least studied among the small millet species and only two reports are available on the genetic diversity of Indian little millet (*P. sumatrense*) on the basis of morphological traits (Arunachalam et al., 2003; Selvi et al., 2015). Molecular markers have already proved their potential in crop improvement and evaluation of genetic resources and among all the dominant markers, random amplification of polymorphic DNA (RAPD) markers are cost effective and have the advantage of detecting genetic diversity simply and quickly (Demeke et al., 1996). The current report is the first involving molecular diversity analysis of Indian little millet genotypes using molecular markers. The only known reference available is M'Ribu and Hilu (1994) who applied RAPD markers to discriminate different *Panicum* species.

Materials and methods

Plant materials

Thirty-two accessions of little millet were obtained from different regions of India under the All India Coordinated Minor

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Millet Improvement Project, Rewa, India (Table 1, Fig. 1). Agronomic and biochemical information of various genotypes has been reported by their respective breeding centers. Chandel et al. (2014) provided details on various genotypes among which JK-8 was characterized as a nutri-rich line with 31.82 parts per million (ppm) Fe and 33.00 ppm Zn. It also has more panicles, stay-green character, tolerance to shoot fly and early flowering with suitability for many growing areas of this crop in India. Cultivar JK-36 with early maturity is suitable for all little millet growing areas if India. Cultivar RLM-37 has high Fe (32.20 ppm) and Zn (32.40 ppm). Cultivar OLM-203 also known as Tarini, is a blast-resistant, grain-smut resistant, non-lodging, non-shattering, long duration, high yielding genotype with superior grain quality widely grown in the Indian states of Andhra Pradesh, Orissa, Bihar, and Tamil Nadu. Cultivar TNAU-91(CO) has profuse tillering, early duration, non-lodging with superior grain quality and is suitable for a double-cropping system under rainfed conditions in southern India. Among various genotypes cv. DHLtMV 36-3 ranks highly with grain yields of approximately 2700 kg/ha (Ganesamurthy, 2015) and tolerance against shoot fly (Birsa Agriculture University, 2015). Genotype DLM 103 is also considered as having resistance against shoot fly (Birsa Agriculture University, 2015).

DNA extraction and quantification

Seeds were grown in a polyhouse to collect fresh leaf samples for genomic DNA isolation. Genomic DNA was isolated using the CTAB protocol (Saghai-Maroof et al., 1984). The quality and quantity of DNA were checked using horizontal submarine gel electrophoresis on 0.8% agarose gel and a Thermo® Nanodrop spectrophotometer (ThermoFisher Scientific, USA). DNA samples were diluted accordingly for RAPD analysis.

Random amplification of polymorphic DNA analysis

Sixty random decamer primers (Operon™ Technologies; Alameda, California) with 60–70% guanine and cytosine (GC) contents were used. The polymerase chain reactions (PCR) were performed in 20 μL reaction mixture containing 100 ng of each genomic DNA, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 400 nM of each RAPD primer and 1 U *Taq* DNA polymerase (Merck; Genei, India). The amplification was carried out in a thermal cycler (Agilent Technologies; India Pvt Ltd). PCR was performed at an initial denaturation at 95 °C for 5 min followed by 45 cycles of 60 s denaturation at 95 °C, 60 s annealing at 37 °C, 2 min extension at 72 °C and a final extension at 72 °C for 7 min. The annealing temperatures were readjusted for each random primer. The amplified products were electrophoresed in 1.4% (weight per volume, w/v) agarose gels at 60 V for 2.15 h; amplification was repeated thrice to confirm the reproducibility of the results; bands were visually scored as 1 for the presence and 0 for the absence of each genotype ($n = 48$) using a gel documentation system (Syngene; USA). The binary data matrix was used to calculate Jaccard's similarity coefficient between pairs of accessions using the Simqual module of NTSYS-pc (Numerical Taxonomy System version 2.1; Rohlf, 1998). These distance coefficients were used to construct a dendrogram using the unweighted pair grouped method arithmetic average (UPGMA) using the sequential agglomerative hierarchical and nested algorithm to determine the genetic diversity and relationships among the accessions.

Results and discussion

Determination of relationships among individuals and populations is an important consideration for genetic conservation and the utilization of plant genetic resources. Molecular markers would

Table 1
List of little millet genotypes and their origin centers.

S.	Genotype	Collection centre	State	Parentage
1.	BL-2	Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur	Chhattisgarh	Pure line selection
2.	BL-4	Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur	Chhattisgarh	Pure line selection
3.	BL-6	Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur	Chhattisgarh	Pure line selection
4.	BL-8	Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur	Chhattisgarh	Pure line selection
5.	BL 41-3	Shaheed Gundadhur College of Agriculture and Research Station, Jagdalpur	Chhattisgarh	Pure line selection
6.	DHLtMV 10-2	Orissa University of Agriculture and Technology, Bhubaneswar	Orissa	Pure line selection
7.	DHLtMV-14-1	Orissa University of Agriculture and Technology, Bhubaneswar	Orissa	Pure line selection
8.	DHLtMV-36-3	Orissa University of Agriculture and Technology, Bhubaneswar	Orissa	Pure line selection
9.	DLM-89	Birsa Agricultural University, Ranchi	Jharkhand	Pure line selection
10.	DLM-103	Birsa Agricultural University, Ranchi	Jharkhand	Pure line selection
11.	JK-8	College of Agriculture, Rewa	Madhya Pradesh	Pure line selection
12.	JK-36	Jawaharlal Nehru Agricultural University, Jabalpur	Madhya Pradesh	Pure line selection
13.	RLM-4-1	College of Agriculture, Rewa	Madhya Pradesh	Local collection
14.	RLM-37	College of Agriculture, Rewa	Madhya Pradesh	Local collection
15.	RLM-39	College of Agriculture, Rewa	Madhya Pradesh	Local collection
16.	RLM-41	College of Agriculture, Rewa	Madhya Pradesh	Local collection
17.	RLM-111	College of Agriculture, Rewa	Madhya Pradesh	Local collection
18.	RLM-118	College of Agriculture, Rewa	Madhya Pradesh	Local collection
19.	RLM-141	College of Agriculture, Rewa	Madhya Pradesh	Local collection
20.	RLM-161	College of Agriculture, Rewa	Madhya Pradesh	Local collection
21.	RLM-162	College of Agriculture, Rewa	Madhya Pradesh	Local collection
22.	RLM-181	College of Agriculture, Rewa	Madhya Pradesh	Local collection
23.	RLM-183	College of Agriculture, Rewa	Madhya Pradesh	Local collection
24.	RLM-186	College of Agriculture, Rewa	Madhya Pradesh	Local collection
25.	RLM-190	College of Agriculture, Rewa	Madhya Pradesh	Local collection
26.	RLM-203	College of Agriculture, Rewa	Madhya Pradesh	Local collection
27.	RLM-224	College of Agriculture, Rewa	Madhya Pradesh	Local collection
28.	RLM-238	College of Agriculture, Rewa	Madhya Pradesh	Local collection
29.	RLM-1367	College of Agriculture, Rewa	Madhya Pradesh	Local collection
30.	OLM-203	Orissa University of Agriculture and Technology, Bhubaneswar	Orissa	Selection from KL 2
31.	TNAU-91 (CO)	Tamil Nadu Agricultural University, Coimbatore	Tamil Nadu	Co 2 × MS 1684
32.	TNAU-159	Tamil Nadu Agricultural University, Coimbatore	Tamil Nadu	Pure line selection

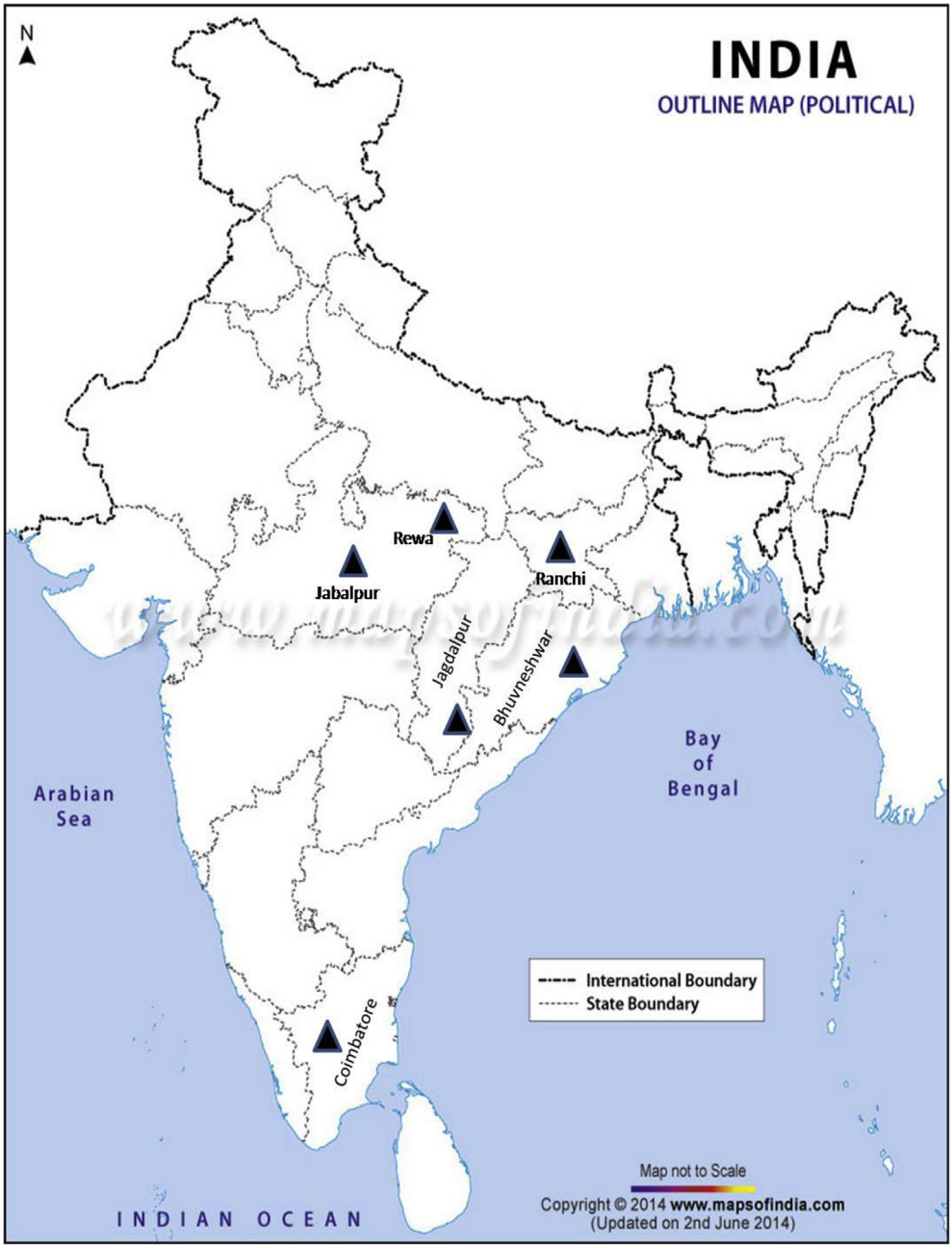

Fig. 1. Little millet collection sites (π) as mentioned in Table 1.

greatly assist breeders to choose superior and diverse parents for crop improvement and speed up the selection of desirable types among a large number of progeny. For the current study, 32 genotypes of *P. sumatrense* were obtained from different regions of India to detect polymorphism using RAPD as the genetic marker. M'Ribu and Hilu (1994) evaluated the potential use of RAPD for studying variations among four indigenous species *of Panicum* and between *P. miliaceum* and *P. sumatrense* obtained from different countries. In both cases, polymorphism in RAPD markers was observed across and within the species. Therefore, RAPD markers can be applied for studying genetic diversity, defining gene pools and identifying cultivars of little millet. During the present study, initially 60 primers were screened among 32 genotypes of *P. sumatrense* and a total of 36 primers were selected on the basis of sharp and clear polymorphic banding pattern for the final analysis. PCR reaction was carried out using a single decamer primer at a time (Table 2). These 36 decamer primers amplified a total of 175 RAPD marker loci. The band size of amplified markers was in the range 100–1900 bp. Maximum numbers of bands (10) were scored by the primer OPH-19 while minimum numbers of bands (2) were scored by the primer OPC-13 (Table 2) with polymorphism information content (PIC) values in the range 0.101 (OPAI-01) to 0.499 (OPE-04). The PIC value of a primer helps in determining its effectiveness in genetic diversity analysis. The high values of PIC indicate the efficiency of the corresponding RAPD markers in the study to evaluate the genetic variability among little millet genotypes. In the current analysis, RAPD marker OPE-04 was more effective having the highest PIC value of 0.499 (Table 2). Data

Table 2
Polymorphism revealed using random amplified polymorphic DNA markers.

S.	Code	Sequence	GC%	TB	Range (~bp)	PB	PP	PIC
1	OPAI-01	5′-CAGGCCCTTC-3′	70	6	550–1750	2	33.33	0.101
2	OPAA-01	5′-AGACGGCTCC-3′	70	6	100–500	5	83.33	0.308
3	OPAA-03	5′-TTAGCGCCCC-3′	70	5	150–450	5	100.0	0.453
4	OPAA-04	5′-AGGACTGCTC-3′	60	7	250–900	4	57.14	0.217
5	OPAB-08	5′-GAAACGGACC-3′	60	6	100–550	5	83.33	0.273
6	OPAB-09	5′-GGGCGACTAC-3′	70	5	120–180	5	100.0	0.431
7	OPAC-12	5′-GGCGAGTGTG-3′	70	5	200–500	5	100.0	0.442
8	OPAC-14	5′-GTCGGTTGTC-3′	60	4	300–600	3	75.00	0.301
9	OPAD-05	5′-ACCGCATGGG-3′	70	6	250–700	6	100.0	0.428
10	OPAD-06	5′-AAGTGCACGG-3′	60	5	550–1450	5	100.0	0.447
11	OPAH-04	5′-CTCCCCAGAC-3′	70	3	700–1000	3	100.0	0.307
12	OPAH-05	5′-TTGCAGGCAG-3′	60	4	400–1000	4	100.0	0.363
13	OPB-02	5' -TGATCCCTGG-3′	60	4	100–200	4	100.0	0.309
14	OPB-18	5′-CCACAGCAGT-3′	60	5	350–1550	2	40.00	0.172
15	OPBB-01	5′-ACACTGGCTG-3′	60	3	250–700	3	100.0	0.359
16	OPBB-04	5′-ACCAGGTCAC-3′	60	6	400–1000	6	100.0	0.489
17	OPBB-05	5′-GGGCCGAACA-3′	70	3	500–900	3	100.0	0.397
18	OPBB-06	5′-CTGAAGCTGG-3′	60	4	200–500	4	100.0	0.199
19	OPBB-07	5′-GAAGGCTGGG-3′	70	3	300–700	3	100.0	0.187
20	OPBB-10	5′-ACTTCGCTGG-3′	60	5	300–1000	5	100.0	0.401
21	OPC-07	5′-GTCCCGACGA-3′	70	4	250–1000	4	100.0	0.191
22	OPC-13	5′-AAGCCTCGTC-3′	60	2	150–250	2	100.0	0.101
23	OPC-14	5′-TGCGTGCTTG-3′	60	4	400–700	2	50.00	0.129
24	OPC-17	5′-TTCCCCCCAG-3′	70	6	110–480	6	100.0	0.454
25	OPC-20	5′-ACTTCGCCAC-3′	60	3	110–350	3	100.0	0.198
26	OPE-04	5′-GTGACATGCC-3′	60	7	190–480	7	100.0	0.499
27	OPE-06	5′-AAGACCCCTC-3′	60	4	150–500	3	75.00	0.202
28	OPF-14	5′-TGCTGCAGGT-3′	60	5	100–470	5	100.0	0.311
29	OPG-II	5′-TGCCCGTCGT-3′	70	4	185–450	4	100.0	0.207
30	OPH-19	5′-CTGACCAGCC-3′	70	10	120–500	8	80.00	0.427
31	OPL-01	5-GGCATGACCT-3′	60	5	100–420	4	80.00	0.317
32	OPL-06	5′-GAGGGAAGAG-3′	60	6	150–450	6	100.0	0.469
33	OPN-06	5′-GAGACGCACA-3′	60	7	400–1120	6	85.71	0.299
34	OPO-03	5′-CTGTTGCTAC-3′	50	5	250–750	5	100.0	0.304
35	OPS-07	5′-TCCGATGCTG -3′	60	4	900–1900	4	100.0	0.266
36.	OPS-19	5′-GAGTCAGCAG-3′	60	4	300–1000	4	100.0	0.231
Total				175	–	155	–	–
Mean				4.86	–	4.31	88.58	0.307

GC = guanine and cytosine; TB = total bands; PB = polymorphic bands; PP = percentage polymorphism; PIC = polymorphism information content.

presented in Table 2 demonstrate the performance of individual RAPD marker used in the study.

Out of the 175 bands amplified using 36 RAPD markers, 20 were monomorphic (12.57%) and the remaining 155 were polymorphic (88.58%). The average number of bands per primer was 4.86 while the average number of polymorphic bands per primer was 4.31. In total, 10 (5.71%) specific bands were amplified by seven primers (Table 3). Among these primers, OPAA-04 amplified a specific band (400 bp) with three genotypes (DLM 103, DHLtMV 36-3, RLM 162).

During the present study, seven markers were observed with unique to different *P. sumatrense* genotypes. These primers can potentially be used as molecular fingerprints to identify genotypes. Primers OPAB-09 (RLM 203), OPBB-01 (BL41-3, RLM 4-1 and TNAU 91), OPBB-04 (JK 36) and OPE-04 (RLM 186) amplified specific bands that were absent in other accessions (data not shown). Primer OPC-17 amplified three specific bands of about 150 bp (RLM 39 and RLM 186), 430 bp (RLM 190, RLM 203 and RLM 224) and 480 bp in (RLM 190, RLM 203 and RLM 224) and primer OPN-06 amplified two specific bands of 400 bp (RLM-37, RLM-161) and 900 bp (RLM-37). Primer OPN-06 can be used to identify RLM-37, a genotype with high Fe (32.20 ppm) and Zn (32.40 ppm) as has also been reported by Chandel et al. (2014). Based on the electrophoretic banding pattern of RAPD primers, pairwise genetic similarity among 32 genotypes for genetic diversity was estimated and a dendrogram was generated using UPGMA (Fig. 2). Cluster analysis indicated that accessions of *P. sumatrense* were in two groups: a major group (Group I) and a minor group (Group II). Group I covered 28 genotypes with two sub-groups, 'A' with 27 genotypes and 'B' with only one genotype (TNAU 159). Subgroup 'A' was further divided into two sub-groups: 'C' containing 26 genotypes and 'F' with only one genotype (RLM-37). In the sub-group 'D', genotypes BL-2, BL-4, BL-6, and BL-8 were clustered together, as these genotypes are the pure line selections developed at Jagdalpur, Chhattisgarh, India. Two genotypes (DHLtMV 10-2, DHLtMV-36-3) had a higher level of similarity falling in the same group as they are from local collections in Bhubaneswar, India. Most of the genotypes collected from Rewa and Jabalpur, India were also clustered in a group. The minor group (Group II) contained four genotypes which were further divided into two sub-groups each comprising two genotypes (RLM-161 and RLM-162 in sub-group 'E' and RLM-203 and RLM-224 in 'F'). These four genotypes are pure line selections of locally collected landraces in Rewa, India. Jaccard's similarity coefficient is a measure of similarity for two sets of data with a range of 0%–100%. The higher the value of Jaccard's coefficient, the greater the similarity between the two genotypes or populations. According to Jaccard's similarity coefficient (Table 3), the highest similarity was observed between BL 41-3 and DHLtMV 10-2 (0.94), while the lowest was between RML 161 and RML 238 (0.29). It is evident from the results of the present analyses that there is a considerable amount of variability among the 32 genotypes originating from the four states in India. The similarity between genotypes collected from two states indicates the exchange of germplasm between them. In accordance with the present study,

Table 3
Jaccard's similarity coefficient among little millet genotypes using random amplified polymorphic DNA markers.

	BL-2	BL-4	BL-6	BL-8	BL 41-3	DHLTMV 10-2	DHLTMV 14-1	DHLTMV 36-3	DLM-89	DLM-103	JK-8	JK-36	RLM 4-1	RLM-37	RLM-39	RLM-41	RLM-111	RLM-118	RLM-141	RLM-161	RLM-162	RLM-181	RLM-183	RLM-186	RLM-190	RLM-203	RLM-224	RLM-238	RLM-1367	OLM-203	TNAU-91	TNAU-159
BL-2	1																															
BL-4	0.89	1.00																														
BL-6	0.88	0.92	1.00																													
BL-8	0.83	0.82	0.88	1.00																												
BL 41-3	0.81	0.79	0.84	0.87	1.00																											
DHLTMV10-2	0.82	0.78	0.84	0.87	0.94	1.00																										
DHLTMV14-1	0.79	0.75	0.78	0.85	0.85	0.88	1.00																									
DHLTMV36-3	0.71	0.69	0.72	0.75	0.79	0.81	0.82	1.00																								
DLM-89	0.77	0.75	0.79	0.83	0.84	0.89	0.85	0.83	1.00																							
DLM-103	0.69	0.66	0.68	0.75	0.76	0.76	0.78	0.80	0.81	1.00																						
JK-8	0.77	0.75	0.79	0.82	0.84	0.87	0.85	0.80	0.89	0.84	1.00																					
JK-36	0.66	0.63	0.62	0.68	0.67	0.69	0.71	0.70	0.73	0.77	0.73	1.00																				
RLM 4-1	0.66	0.64	0.69	0.73	0.80	0.81	0.77	0.77	0.83	0.81	0.85	0.70	1.00																			
RLM-37	0.66	0.55	0.57	0.61	0.60	0.64	0.69	0.66	0.68	0.67	0.67	0.68	0.68	1.00																		
RLM-39	0.70	0.65	0.67	0.73	0.69	0.73	0.74	0.68	0.77	0.71	0.74	0.66	0.70	0.64	1.00																	
RLM-41	0.76	0.72	0.75	0.78	0.81	0.84	0.83	0.81	0.88	0.84	0.86	0.80	0.83	0.73	0.80	1.00																
RLM-111	0.59	0.56	0.58	0.66	0.65	0.68	0.70	0.73	0.70	0.71	0.70	0.61	0.71	0.64	0.64	0.75	1.00															
RLM-118	0.66	0.65	0.66	0.73	0.72	0.75	0.75	0.74	0.77	0.80	0.80	0.74	0.82	0.73	0.69	0.84	0.74	1.00														
RLM-141	0.66	0.63	0.67	0.76	0.74	0.76	0.77	0.72	0.76	0.77	0.81	0.68	0.79	0.67	0.69	0.78	0.70	0.85	1.00													
RLM-161	0.36	0.37	0.37	0.39	0.34	0.35	0.38	0.35	0.35	0.37	0.36	0.36	0.35	0.33	0.34	0.34	0.32	0.38	0.40	1.00												
RLM-162	0.41	0.40	0.41	0.45	0.44	0.44	0.43	0.42	0.43	0.44	0.42	0.46	0.41	0.34	0.46	0.44	0.38	0.43	0.40	0.62	1.00											
RLM-181	0.71	0.66	0.69	0.75	0.74	0.79	0.77	0.72	0.79	0.75	0.79	0.73	0.74	0.67	0.74	0.81	0.68	0.75	0.72	0.35	0.51	1.00										
RLM-183	0.73	0.70	0.73	0.78	0.79	0.82	0.81	0.81	0.85	0.82	0.84	0.76	0.80	0.69	0.75	0.86	0.70	0.83	0.80	0.35	0.48	0.83	1.00									
RLM-186	0.67	0.64	0.64	0.69	0.68	0.71	0.75	0.73	0.74	0.73	0.74	0.68	0.72	0.72	0.68	0.74	0.69	0.77	0.74	0.36	0.40	0.70	0.84	1.00								
RLM-190	0.59	0.56	0.57	0.62	0.61	0.64	0.66	0.68	0.64	0.69	0.68	0.64	0.64	0.62	0.62	0.67	0.65	0.73	0.71	0.41	0.44	0.67	0.73	0.80	1.00							
RLM-203	0.49	0.47	0.45	0.47	0.43	0.46	0.47	0.47	0.47	0.49	0.47	0.48	0.46	0.46	0.48	0.51	0.48	0.53	0.49	0.53	0.50	0.49	0.51	0.54	0.63	1.00						
RLM-224	0.39	0.39	0.35	0.39	0.39	0.37	0.40	0.39	0.42	0.43	0.39	0.43	0.38	0.32	0.42	0.43	0.40	0.39	0.38	0.39	0.49	0.42	0.42	0.40	0.43	0.53	1.00					
RLM-238	0.66	0.64	0.65	0.72	0.69	0.71	0.75	0.74	0.76	0.73	0.79	0.71	0.73	0.67	0.68	0.78	0.70	0.77	0.68	0.29	0.39	0.73	0.79	0.77	0.65	0.49	0.43	1.00				
RLM-1367	0.74	0.70	0.73	0.79	0.78	0.80	0.76	0.76	0.83	0.80	0.84	0.72	0.76	0.66	0.75	0.83	0.69	0.78	0.74	0.33	0.43	0.79	0.81	0.72	0.68	0.49	0.41	0.81	1.00			
OLM-203	0.69	0.67	0.68	0.72	0.73	0.74	0.74	0.73	0.74	0.74	0.76	0.65	0.69	0.65	0.70	0.77	0.69	0.75	0.67	0.30	0.40	0.72	0.76	0.71	0.65	0.49	0.40	0.81	0.83	1.00		
TNAU-91	0.68	0.68	0.71	0.71	0.74	0.76	0.72	0.71	0.77	0.74	0.80	0.66	0.71	0.61	0.70	0.79	0.64	0.72	0.69	0.32	0.43	0.78	0.78	0.68	0.67	0.48	0.38	0.74	0.85	0.79	1.00	
TNAU-159	0.58	0.60	0.58	0.59	0.59	0.62	0.65	0.59	0.61	0.62	0.65	0.64	0.62	0.59	0.59	0.67	0.59	0.68	0.65	0.36	0.40	0.62	0.69	0.68	0.65	0.53	0.39	0.69	0.64	0.61	0.69	1.00

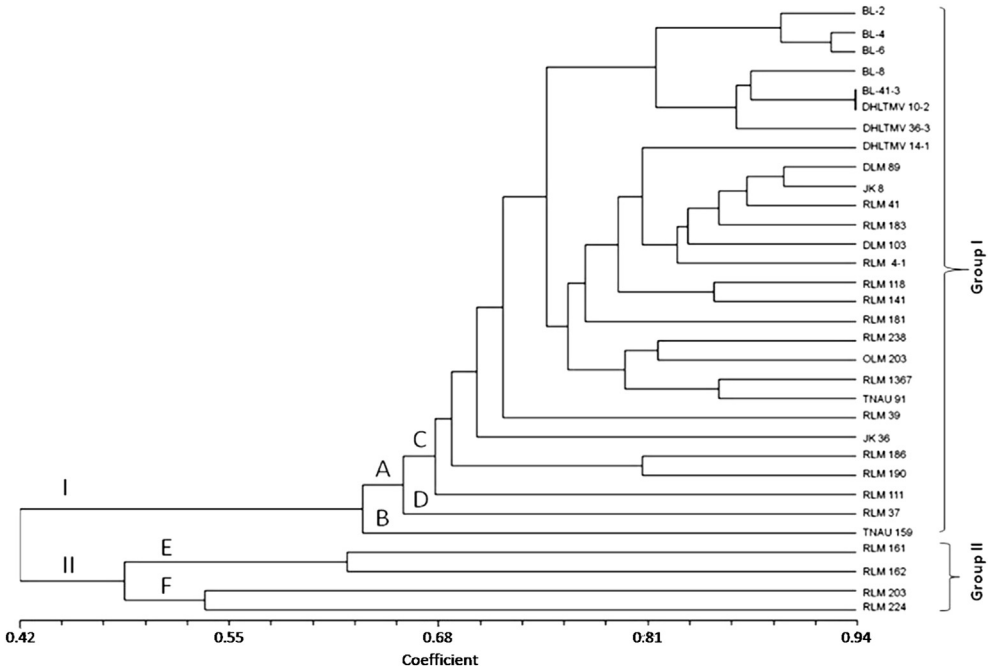

Fig. 2. Dendrogram showing relationship among little millet (*Panicum sumatrense*) genotypes based on random amplified polymorphic DNA analysis.

similar results have been reported in foxtail millet diversity using molecular markers (Kumari et al., 2011).

In the two-dimensional scaling, two groups of genotypes were formed (Fig. 3). Most of the genotypes followed similar grouping as in Fig. 1. Genotype RLM-141 showed different grouping in both figures which may have been due to the low number of markers used in the study. This also confirmed the importance of a higher number of loci and their coverage of the entire genome for presenting consistent estimation of genetic relatedness among genotypes (Gupta et al., 2008).

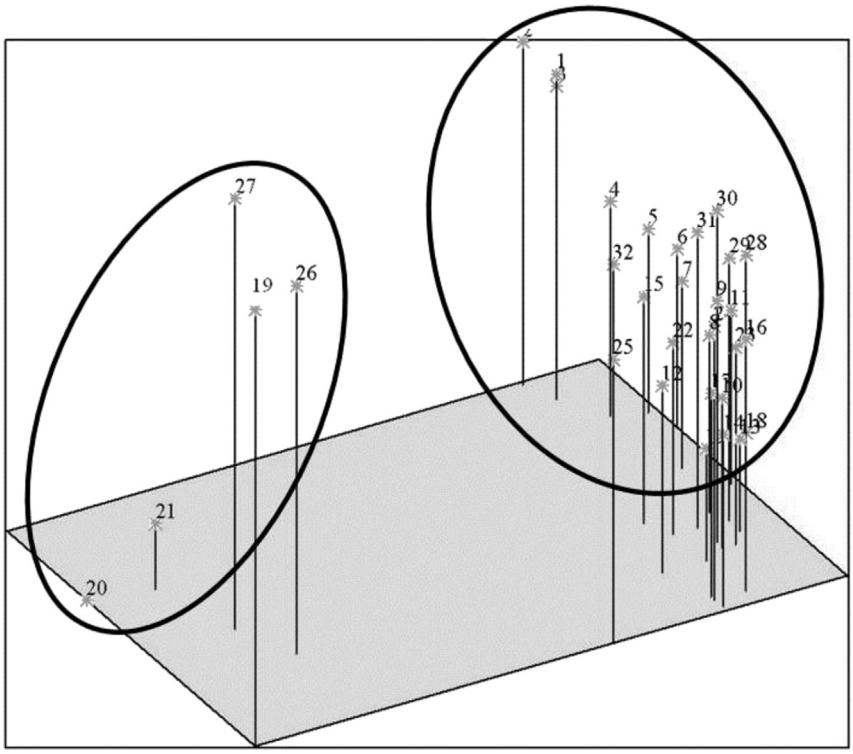

Fig. 3. Two-dimensional scaling of little millet (*Panicum sumatrense*) genotypes based on random amplified polymorphic DNA analysis, where numbers represent serial no. of genotypes as mentioned in Table 1.

This is the first report involving molecular diversity analysis of a large set of little millet genotypes. Grouping of the genotypes according to their collection centers reflects the presence of a higher level of genetic diversity among genotypes collected from geographically distinct areas. The overall genetic diversity of a taxon has great implications for its long-term survival and evolution. Therefore, knowledge of the degree and pattern of genetic diversity is important for designing conservation strategies.

A high level of genetic diversity among *P. sumatrense* genotypes was observed using the RAPD markers. The findings can help in the identification of diverse parents for genetic improvement of *P. sumatrense* using hybridization programme. Such a high level of diversity is a reflection of adaptation to the environment, which is beneficial to propagation, resource conservation, the domestication of wild species and the screening of a specified geographic area.

Conflict of interest

The authors declare that they have no competing interests.

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