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Genetic Diversity Evaluation of *Thamnocalamus spathiflorus* (Trin.) Munro Accessions through Morphological and Randomly Amplified Polymorphic DNA (RAPD) Markers

Chandrakant Tiwari[1,*], Meena Bakshi[2] and Dinesh Gupta[3]
[1]*Department of Forest Biology & Tree Improvement, College of Forestry, Banda University of Agriculture & Technology (BUAT), Banda 210001, India*
[2]*Plant Physiology Discipline, Botany Division, Forest Research Institute, Dehradun 248006, India*
[3]*Department of Silviculture and Agroforestry, College of Forestry, Banda University of Agriculture & Technology (BUAT), Banda 210001, India*

Abstract

Biodiversity refers to the total number and variation among species of flora and fauna of an area. Due to tremendous biotic especially anthropogenic pressure these natural resources are being vanishing. In present study genetic diversity among accessions of *Thamnocalamus spathiflorus* was evaluated. A total of 51 vegetative characters and 42 primers (10-mer) were screened. Out of 42 screened primers, 28 polymorphic primers were selected for further analysis. A total of 263 bands were recorded as polymorphic whereas 48 bands were monomorphic. The resolving power (Rp) of 28 Randomly Amplified Polymorphic DNA (RAPD) primers ranged from 4.6 (OPE08) to 17.6 (OPA11). The polymorphic information content (PIC) value ranged from 0.21 (OPAH09) to 0.44 (OPG02). The result revealed high degree of genetic relatedness (56 to 80%). Cluster analysis revealed two major clusters both for morphology as well as RAPD. Unlike morphological characterization, the accession (D5) from Bahli, Rampur, Shimla (H.P.) was clustered separately from the others in RAPD cluster analysis. Accessions with closed locality grouped together through RAPD marker system however analogy was recorded for morphological traits. The study conducted reflects the utility of RAPD technique for species identification and phylogenetic studies in bamboo for conducting bamboo breeding program.

Key Words: polymorphism, randomly amplified polymorphic DNA (RAPD), polymorphic information content (PIC) value, *Thamnocalamus spathiflorus*, cluster analysis

Introduction

Biodiversity represents the richness of flora and fauna in a particular region. The diminution of natural resources is being increasing at alarming rate which leads to the wiping out the forest and diversity of various flora and fauna day by day. This necessitates to strategies the protection and preservation of our natural resources.

Among various conservation programmes, evaluation of germplasm plays a crucial role in conserving a species. Importance of germplasm characterization on the basis of morphological and genetic characteristics of a species was

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Corresponding author: Chandrakant Tiwari
Department of Forest Biology & Tree Improvement, College of Forestry, Banda University of Agriculture & Technology (BUAT), Banda 210001, India
Tel: +91-5192-232305, Fax: +91-6144-4472, E-mail: tiwari.chandrakant08@gmail.com

suggested by Generoso et al. (2016).

Traditional identification of plant was based on morphological characters like floral morphology, growth habit etc. which show high degree of variability due to climatic conditions in which they thrive. Diversity evaluation and long term conservation programme can be achieved on sustainable level through application of molecular techniques. Literature on application of molecular techniques for the study of genetic diversity in bamboo is very limited. Restriction Fragment Length Polymorphisms (RFLP) was used in *Phyllostachys* by Friar and Kochert (1991; 1994), phylogeny studies of Asian bamboos was carried out by Watanbe et al. (1994) and phylogeny of world bamboos was done by Kobayashi (1996). Application of molecular technique along with morphological parameters can provide better results than the morphology alone (Campbell et al. 1995).

Among various molecular techniques evolved, Randomly Amplified Polymorphic DNA (RAPD) assay is the most cost effective and less time consuming method for characterization of genotypes. Moreover, very low amount of DNA is required for RAPD assay. Utility of RAPD maker in bamboo conservation, breeding and management programme was studied by Konzen et al. (2017). Phylogenetic studies on various grasses including bamboo were carried out (Huff et al. 1993; Gunter et al. 1996; Kölliker et al. 1999; Nair et al. 1999; Tiwari et al. 2015).

Thamnocalamus spathiflorus (Tham Ringal) is a dwarf (hill) bamboo inhabitant of Garhwal Himalaya at an altitude of 2400 m-3000 m. Like other bamboos, *T. spathiflorus* also has erratic and long flowering cycles. *T. spathiflorus* flowers both sporadically and gregariously. The gregarious flowering was reported in 2001-2002 throughout the Uttarakhand State, India. Death of clumps after seed setting has been reported by Naithani et al. (2003). Gregarious flowering coupled with death of clump leads to threat on survival of the species. Moreover, the species is being used by the inhabitant extensively which resulted to vanishing of these resources from the natural forest. These activities resulted in complete wiping out of the species in some of the area which had thick cover of the species. Circumstances necessitates the conservation of the species. In view of the above, information on the phylogeny and genetic diversity of available germplasm is essential for the identification of potential germplasm groups and for optimizing hybridization and selection procedures. In present study, RAPD markers along with morphology were chosen to study the genetic variation and to determine the genetic diversity within species.

Materials and Methods

Experimental site

The present investigations were carried out in the germplasm of hill bamboos (Ringal) established at Khirsu, Pauri (Garhwal), Uttarakhand, India (30°10.368' N latitude,

Table 1. Geographical details and genomic DNA of different genotypes of *Thamnocalamus spathiflorus* in Hill Bamboo Germplasm at Khirsu (India)

Accession. No.*	Place of Collection*	DNA Conc. (ng/μL)	Ratio of absorbance at 260/280 nm ($A_{260/280}$)
D1.	Chopta forest I, Chamoli	84.20	1.90
D2.	Musk deer farm I, Chopta, Chamoli	555.90	1.77
D3.	Musk deer farm II Chopta, Chamoli	806.30	1.75
D4.	Thamrikund I, Munsyari, Pithoragarh	572.70	1.78
D5.	Bahli, Rampur, Shimla (H.P.)	775.30	1.80
D6.	Chopta II forest, Chamoli	615.60	1.77
D7.	Thamrikund II, Munsyari, Pithoragarh	275.20	1.65
D8.	Thamrikund III, Munsyari, Pithoragarh	292.10	1.78
D9.	Thamrikund IV, Munsyari, Pithoragarh	509.10	1.61
D10.	Taklech, Rampur, Shimla (H.P.)	630.10	1.77

*Source: National Bamboo Mission Project Report (2011).

78°52.167' E longitude and 1,934 m altitudes above sea level).

Experimental material

Ten accessions of *Thamnocalamus falconeri* were selected and tagged in Hill Bamboo Germplasm. The accessions selected for study represented diverse locations of Uttarakhand and Himanchal Pradesh. The Table 1 illustrates the geographical locations of collection sites.

Morphological study

Several taxonomic descriptors as morphological traits were chosen for scoring the accessions. Each accessions were taken as independent operational taxonomic unit (OTU) and mean values from three independent replications were used as representative data for each of the quantitative morphological descriptors. A total of twenty quantitative characters and thirty one qualitative characters were priorities as morphological descriptors for all the OTU's.

Genomic DNA isolation and RAPD study

Plant materials

The plant material i.e. young leaves were collected from the study site i.e. Hill Bamboo Germplasm, Khirsu, Garhwal (India). The study site is represented by 30° 10.368' N latitude, 78° 52.167' E longitude and 1,934 m altitudes above sea level. The selected accessions were tagged properly. The collected leaf samples were collected in polybags and stored in ice bucket and brought at Plant Physiology Laboratory (FRI, Dehradun, India). The leaves were stored at -20°C in freezer (vest frost DFS 345) for further analysis.

Plant DNA extraction

Total genomic DNA was extracted by CTAB method developed by Stange et al. (1998) from the leaves of ten selected accessions and as per nature of plant species under study, necessary modifications were done in the standard protocol. Quality of extracted genomic DNA sample was checked at 1% agarose gels and quantified by using Biophotometer ($A_{260/280}$). The purity of DNA sample was determined in the range between 1.61-1.90 and quantity of extracted DNA ranged from 84.20-806.30 ng/μL (Table 1).

RAPD analysis

The reaction mixture (25 μL) containing10X Taq buffer, 2 mM dNTPs, 2.5 mM $MgCl_2$, Taq Polymerase (5 U/μL), 20 mM Primer and 50 ng/μL genomic DNA was prepared. The RAPD- PCR analysis was performed in a thermal cycler (Techne, FPROG05D) with a cycling program of intial denaturation 94°C for 4 min., initiation 94°C for 1 min., elongation 37°C for 1min. and finally termination at 72°C for 2 min for 45 cycles. Final extention was done at 72°C for 5 min. The agarose 1.5% (w/v) gel electrophoresis was used for the separation of amplified (PCR) products.

Statistical analysis

Morphological analysis

Morphological data was kept under one-way ANOVA (Analysis of variance) by using Genstat version 3.2. The

Table 2. Dissimilarity matrix of 10 accessions of *Thamnocalamus spathiflorus* (Morphology)

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
D1	0.00									
D2	24.59	0.00								
D3	3.75	22.88	0.00							
D4	5.90	25.42	7.42	0.00						
D5	21.39	26.30	23.53	19.81	0.00					
D6	15.18	10.48	13.28	16.26	23.61	0.00				
D7	11.86	26.10	14.73	10.94	10.60	19.61	0.00			
D8	5.41	20.52	5.30	6.93	20.09	11.06	11.90	0.00		
D9	21.95	13.13	22.17	21.48	14.57	14.48	17.50	18.32	0.00	
D10	15.46	13.07	15.31	15.13	14.73	9.60	13.53	11.51	7.85	0.00

Table 3. Morphological parameters (quantitative) of different accessions of *T. spathiflorus*

Accession	Clump circumference (cm)	Number of culms	Number of young shoots	Culm height (cm)	Culm diameter (cm)	Culm to culm distance (cm)	Internodal length (cm)	Number of internodes per culm	Leaf length (cm)	Leaf width (cm)
D1	190.00	14.00	11.00	122.67	0.32	3.50	13.67	11.67	2.74	0.26
D2	210.00	7.00	5.00	43.00	0.19	3.17	10.33	8.00	1.96	0.30
D3	70.00	16.00	2.33	103.83	0.23	3.33	10.50	12.67	2.57	0.27
D4	210.00	17.33	18.00	57.47	0.29	4.33	7.33	10.00	1.84	0.37
D5	140.00	32.00	23.00	170.83	0.54	3.50	12.50	12.67	3.00	0.29
D6	110.00	8.00	4.00	12.50	0.18	3.00	13.00	10.00	2.09	0.26
D7	110.00	25.00	17.00	75.17	0.31	2.33	11.50	12.33	2.16	0.34
D8	180.00	9.00	2.67	42.83	0.30	3.67	13.43	14.67	2.36	0.19
D9	40.00	16.00	7.00	19.17	0.38	2.67	12.17	5.00	2.20	0.34
D10	198.33	13.00	6.00	21.00	0.14	2.67	11.33	7.33	2.05	0.33
Mean	145.83	15.73	9.60	66.85	0.29	3.22	11.58	10.43	2.30	0.30
Sign.	***	***	***	***	NS	NS	***	***	NS	NS
C.D.	11.21	3.38	2.06	58.67	0.28	1.18	2.14	3.14	0.81	0.10

Accession	No. of nodes per branch	Number of leaves per node	Bud length (cm)	Bud width (cm)	Sheath length (cm)	Sheath breadth (cm)	Blade length (cm)	Total sheath length/ breadth at base	Total sheath length/ blade length	Culm sheath area (cm^2)
D1	10.00	11.67	0.29	0.60	7.63	1.93	1.13	3.98	6.90	10.70
D2	6.67	10.67	0.15	0.25	5.93	1.27	0.97	4.71	6.31	6.62
D3	14.33	11.33	0.04	0.51	7.43	1.07	0.47	7.18	17.70	7.44
D4	8.33	6.33	0.15	0.40	11.67	2.00	0.30	5.83	38.89	14.91
D5	11.33	20.67	0.06	0.50	7.00	2.00	0.72	3.50	9.73	9.07
D6	8.67	11.33	0.15	0.49	11.40	2.07	0.87	5.77	15.91	21.40
D7	5.33	4.00	0.10	0.50	10.40	2.33	1.07	4.58	10.63	16.445
D8	3.67	2.00	0.06	0.40	11.90	2.57	1.07	4.62	11.69	24.38
D9	4.33	4.00	0.06	0.16	7.17	1.30	1.03	5.58	6.94	8.93
D10	4.67	3.33	0.20	0.49	5.63	1.57	0.67	3.88	8.65	5.44
Mean	7.73	8.53	0.13	0.43	8.62	1.81	0.83	4.96	13.33	12.53
Significance	***	***	***	***	***	***	NS	NS	***	***
C.D.	2.47	2.60	0.02	0.03	1.76	0.51	0.45	1.76	6.60	5.49

***Significance at 0.1%.

Table 4. Morphological parameters (qualitative) of different accessions of *T. spathiflorus*

Accession	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Clump density	2	1	0	1	1	0	0	2	1	0
Clump habit	2	0	0	3	0	0	0	2	1	0
Bending of culm	2	1	0	3	0	0	0	2	1	0
Culm colour	0	1	0	3	0	0	0	2	1	0
Culm surface	3	1	1	3	0	0	0	2	1	0
Leaf colour/dorsal	3	0	0	3	0	0	0	2	1	0
Leaf colour/ventral	3	1	0	3	0	0	0	2	1	0
Leaf habit	1	1	0	3	1	0	0	2	1	0
Leaf stalk	3	0	0	0	0	0	0	2	1	0
Leaf surface	1	0	0	3	0	0	0	2	1	0
Leaf texture	2	1	0	1	1	0	0	2	1	0
Internodal shape	2	0	0	3	0	0	0	2	1	0
Bud position	2	1	0	3	0	0	0	2	1	0
Bud shape	0	1	0	3	0	0	0	2	1	0
Bud number	3	1	1	3	0	0	0	2	1	0
Swollen node	3	0	0	3	0	0	0	2	1	0
Nodal sheath scar	3	1	0	3	0	0	0	2	1	0
Curved lower nodal branches	1	1	0	3	1	0	0	2	1	0
Piercing culm sheath	1	1	0	0	1	0	0	1	2	0
Ciliate margin	1	1	0	0	1	0	0	1	2	0
Pubescent adaxial hair	1	1	0	0	1	0	0	1	2	0
Pubescent abaxial hair	1	1	0	0	1	0	0	1	2	0
Blade reflexed	1	1	0	0	0	0	0	1	2	0
Hairy margin on blade	1	1	0	0	0	0	0	1	2	0
Ligule margin	1	1	1	0	0	0	0	1	2	0
Hairs on ligule	1	1	1	0	0	0	0	1	2	0
Bud arrangement	1	1	0	0	1	0	0	1	2	0
Auricle	1	1	0	0	0	0	0	1	2	0
Culm sheath texture	1	1	0	0	1	0	0	1	2	0
Culm sheath shape	1	1	0	0	1	0	0	1	2	0
Variable sheath Size	1	1	0	0	1	0	0	1	2	0

F-value at 0.1% level of significance was compared with the tabulated values and respective degrees of source and error and critical difference (CD) or least significant difference (LSD) were calculated for respective data.

RAPD profile data

The amplified bands obtained was recorded in a binary quantitative matrix as 1 (band present) and 0 (band absent). Only reproducible amplified fragments were scored whereas weak bands of negligible intensity and smeared bands were excluded from final data analysis. Resolving power of the primer (Rp) of RAPD primers were determined (Prevost and Wilkinson 1999) and polymorphic information content (PIC) values for each of the amplified primers were determined by using the formula of Liu and Furnier (1993).

Cluster analysis

Morphology study included observation of total fifty one morphological descriptors (18 cum sheath and 33 culm descriptors) for each of the ten OTU's. Mean values from three independent replications were used as OTU representative data for each of the quantitative morphological descriptors. Dendrogram was constructed on the basis of scored qualitative and quantitative data. RAPD variations were estimated on the basis of Jaccard's similarity index and

UPGMA clustering. Each accession was considered as a taxonomical operational unit (OTU) and RAPD data were transformed into a binary matrix. The Jaccard's distance matrices generated by RAPD using NTSYS-PC 2.11 and Darwin (version 5.0) software programme were used to construt dendrogram with bootstrap values.

Results

Morphological variability

The quantitative as well as qualitative data pertaining to morphological variations among accessions of *T. spathiflorus* are cited in Tables 2 and 3. Variation among different accessions of *T. spathiflorus* for clump circumference was quite significant. The accession with maximum clump circumference was observed in D2 & D4 (210.00 cm) from Musk deer farm I, Chopta, Chamoli and Thamrikund I, Munsyari, Pithoragarh respectively. Lowest was recorded in D9 (40.00 cm) from Thamrikund IV, Munsyari, Pithoragarh, Chopta. The accession displaying the highest average culm number was D5 (41.00) from Bahli, Rampur, Shimla (H.P.) followed by D7 (25.00) from Thamrikund II, Munsyari, Pithoragarh. The minimum average number of culms was observed in accession D2 (7.00) from Musk deer farm I, Chopta, Chamoli. The details of morphological character of all genotypes is tabulated in Table 2. The overall performance for the characters clump circumference, no. of culms, no. of young shoots, culm height and culm diameter was maximum in genotype D5 (366.37) from Bahli, Rampur, Shimla (H.P.) followed by D1 (337.39) from Chopta forest I, Chamoli. A wide range of significant variations were observed in culm sheath characteristics except blade length and total sheath length/ breadth at base.

Cluster analysis

Dissimilarity matrix obtained using Jaccard's coefficient revealed that similarity was ranged between 73.70 to 96.25% (Table 4). The whole dendrogram was split into two major clusters. Cluster I comprised of total six accessions viz., D3, D1, D8, D4, D7 and D5, belong to Musk deer farm II , Chopta forest I, Thamrikund III, Thamrikund I, Thamrikund II and Bahli, Rampur respectively. Cluster II comprised of two accessions only i.e. D6, D2, D10 and D9 from Chopta II forest, Musk deer farm I, Taklech, Rampur and Thamrikund IV respectively (Fig. 1). Maximum similarity (96.25%) was found for the accessions D1 and D3 and minimum (73.70%) between the accessions D2 and D5.

RAPD analysis

Initially, forty two RAPD primers were screened but only twenty eight were found polymorphic, hence they were kept for further analysis. Polymorphism was recorded with 263 bands whereas 48 bands were categorized into mono-

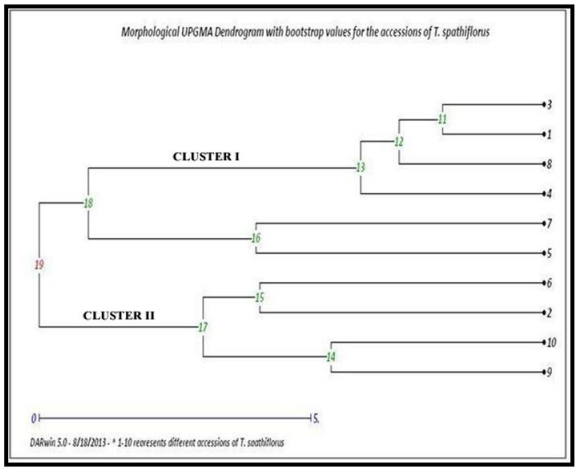

Fig. 1. Morphological UPGMA dendrogram with bootstrap values for the accessions of *T. spathiflorus.*

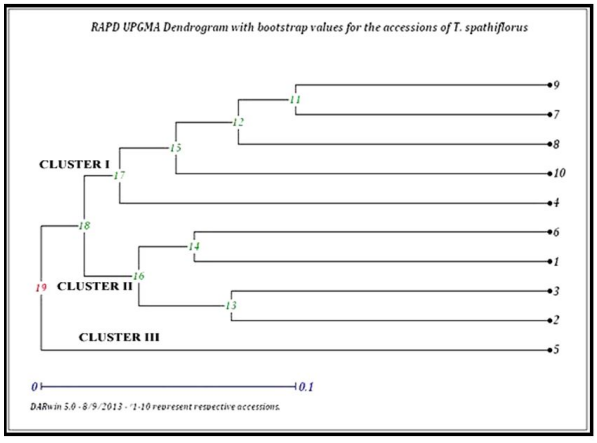

Fig. 2. RAPD UPGMA dendrogram with bootstrap values for the accessions of *T. spathiflorus*.

Table 5. RAPD primers with sequences and the properties of amplified products per genotype in *T. spathiflorus*

Primer	Primer seq. (5'–3')	TNB	NPB	NMB	P %	Rp	PIC
Primer-1	5'GTGAGGCGTC3'	12	11	1	91.67	12.8	0.31
Primer-2	5'ACTCAGCCAC3'	13	11	2	84.62	11.91	0.34
Primer-3	5'GGGGGTTAGG3'	13	13	0	100.0	16.0	0.37
OPE01	5'CCCAAGGTCC3'	8	7	1	87.50	10.2	0.43
OPE08	5'TCACCACGGT3'	8	8	0	100.0	4.6	0.34
OPF04	5'GGTGATCAGG3'	15	12	3	80.00	15.6	0.36
OPF06	5'GGGAATTCGG3'	10	10	0	100.0	10.2	0.38
OPG02	5'GGCACTGAGG3'	13	10	3	76.92	16.0	0.44
OPG12	5'CAGCTCACGA3'	10	10	0	100.0	8.0	0.31
OPH04	5'GGAAGTCGCC3'	9	8	1	88.89	12.8	0.38
OPH09	5'TGTAGCTGGG3'	11	8	3	72.72	14.2	0.36
OPL07	5'CAGCGACAAG3'	14	10	4	71.43	16.8	0.26
OPL18	5'TGCCCAGCCT3'	10	9	1	90.00	10.6	0.25
OPAE04	5'CCAGCACTTC3'	9	8	1	88.89	13.6	0.39
OPAE07	5'GTGTCAGTGG3'	14	11	3	78.57	15.4	0.30
OPAH03	5'GGTTACTGCC3'	10	7	3	70.00	11.4	0.29
OPAH09	5'AGAACCGAGG3'	15	12	3	80.00	13.0	0.21
OPN04	5'GACCGACCCA3'	13	13	0	100.0	16.4	0.36
OPN06	5'GAGACGCACA3'	9	9	0	100.0	12.2	0.32
OPQ06	5'CCGTCGGTAG3'	6	5	1	83.33	6.8	0.37
OPR07	5'ACTGGCCTGA3'	11	8	3	72.73	15.2	0.35
OPR08	5'CCCGTTGCCT3'	10	7	3	70.00	14.6	0.29
OPA04	5'AATCGGGCTG3'	9	6	3	66.67	10.8	0.35
OPA17	5'GACCGCTTGT3'	8	7	1	87.50	9.8	0.35
OPA20	5'GTTGCGATCC3'	12	10	2	83.33	15.6	0.29
OPA11	5'TCGCCGCAAA3'	17	13	4	76.47	17.6	0.30
OPA19	5'GTCCGTACTG3'	11	10	1	90.91	9.6	0.34
OPA20	5'GTGGCTCCGT3'	11	10	1	90.91	15.8	0.38
Total		311	263	48	-	-	-
Average		11.11	9.39	1.71	85.11	12.78	0.34

TNB, total number of bands; NPB, number of polymorphic bands; NMB, number of monomorphic bands; P%, polymorphism percentage; Rp, resolving power; PIC, polymorphic information content.

morphic (Fig. 2). Six RAPD primers viz. Primer-3, OPE08, OPF06, OPG12, OPN04 and OPN06 showed 100% polymorphism. Minimum polymorphism (70.00%) was recorded for OPAH03. The resolving power (Rp) of the 28 RAPD primers used for the analysis ranged from 4.6 (OPE08) to 17.6 (OPA11) with an average of 12.78 per primer. Polymorphic information content ranged from 0.21 (OPAH09) to 0.44 (OPG02) with an average value of 0.34 per primer (Table 5).

Cluster analysis

Data scored from 10 accessions of *T. spathiflorus* with 28 RAPD primers, were used to generate similarity coefficients. The genetic relatedness among the accessions revealed by Unweighted pair group methods with arithmetic mean (UPGMA) cluster analysis is presented in Table 6. The dendrogram revealed two major clusters at 60% similarity level. First major contained nine accessions out of ten which were further categorized into two sub- clusters and four micro- clusters. Nine accessions of the first cluster comprised of D1, D2, D3, D4, D6, D7, D8, D9 and D10 at overall similarity of 68% representing different localities (Table 1) whereas second major cluster comprised of only one accession i.e. D5. Similarity between different accessions was

Table 6. Similarity matrix index is showing relatedness among the accessions of *T. spathiflorus* using RAPD marker

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
D1	1.00									
D2	0.71	1.00								
D3	0.66	0.75	1.00							
D4	0.57	0.64	0.70	1.00						
D5	0.59	0.62	0.62	0.62	1.00					
D6	0.72	0.63	0.70	0.64	0.64	1.00				
D7	0.61	0.62	0.66	0.60	0.57	0.63	1.00			
D8	0.60	0.62	0.68	0.71	0.58	0.66	0.72	1.00		
D9	0.60	0.60	0.66	0.68	0.59	0.64	0.80	0.79	1.00	
D10	0.62	0.61	0.67	0.65	0.56	0.65	0.71	0.67	0.73	1.00

laid between 56 to 80%. Minimum similarity (56%) was found between accessions D1 and D5 followed by D1 to D4 and D5 to D6 (57%). Maximum (80 %) similarity was found between accessions D7 and D9 (Fig. 3).

Discussion

Variation is the pre requisite to be investigated for hereditary change in any species and subsequently assume a key part in plant breeding programs (Burley and Styles 1976; Zobel and Talbert 1984; Tewari 1992). Essential aspects, for example, determination, hybridization can be utilized to control the inconstancy in an organic populace. Information of hereditary fluctuation exhibit in accessible germplasm, kind of hereditary relationship between different traits and genotype x condition associations is basic for picking fitting plant material for determination. Variations inside an animal are because of impacts of heredity and condition. Natural impacts can be diminished by developing the indistinguishable genotypes under uniform climatic and site conditions.

To access variability within and among various accessions of four Ringal species, the present work was carried out in uniform environmental conditions in a Germplasm raised at Khirsu, Pauri (1934 m) in the year 2008 under National Bamboo Mission project. All the species prevailed well at this altitude.

Two methodologies could be taken when estimating contrasts among people, populaces or species. The main approach "morphological variation" which reflects characters influenced by environment and is fundamental for evaluation and phenotypic determination in any plant breeding program. Since phenotypic varieties are the total of natural and in addition hereditary collaborations, this approach would be less proper to give legitimate data to delineate the phylogeny. Subsequently, the second approach "genetic variation", is fundamental to identify hereditary contrasts and hereditary similitude between sets of firmly related species, geological races or ecotypes if developed under indistinguishable condition.

Development in any tree species is the net after effect of collection of photosynthates which is exceptionally helpful and substantial device for anticipating the development execution of the plants. Numerous characters differ inside the species however few demonstrate helpful for intraspecific gathering (Kupicha 1976).

The findings recorded vital interspecies furthermore as intraspecific variations with relevancy morphological traits. Mean number of culms imparted significant variations for all species. The new young shoots of bamboo has large biological process price utilized in cuisines and decoctions for numerous remedial functions (Kapur 1990). Variability in young shoot growth was reported by Kleinhenz and Midmore (2001). Rasool (2011) studied variability for this character in twenty accessions of *D. strictus*. Since, Ringal is employed extensively for handicrafts and ideally chosen with long internodes thus, internodal length forms a valuable character. There was a big variation in internodal length in *T. spathiflorus* genotypes. Internodal variability at population level was discovered by Bhattacharya et al. (2009) in *T. spathiflorus* subsps. *spathiflorus*.

Based on assessable quantitative traits of interest viz. clump circumference, number of culms/clump, number of

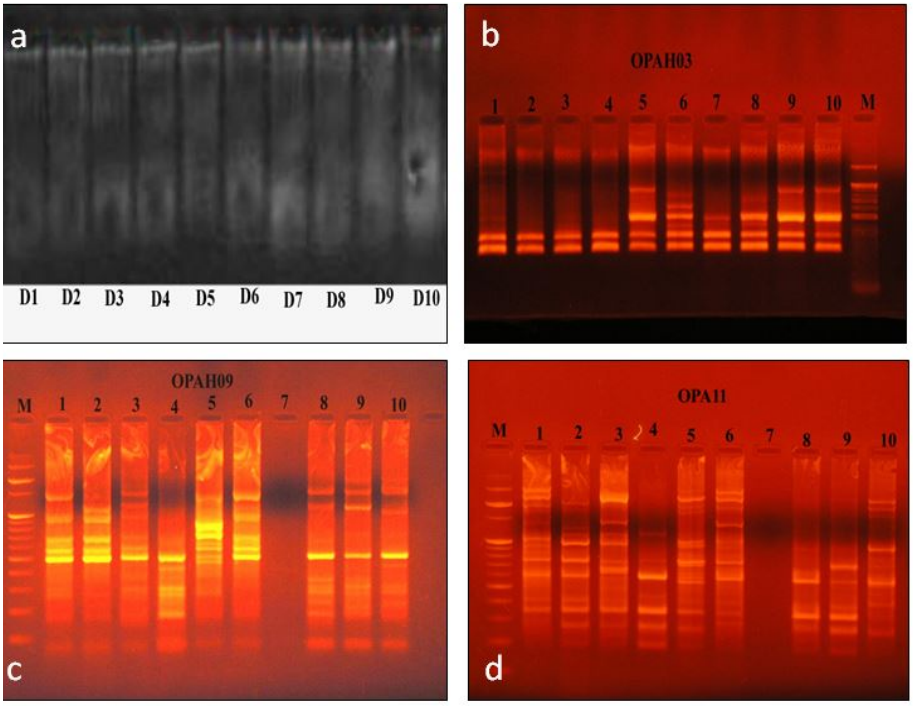

Fig. 3. a, b, c, d, represent genomic DNA and PAPD amplification with the primers OPR07, OPA04 & OPA17 respectively of accessions of *T. spathiflorus*.

young shoots, culm height and culm diameter, accessions D5 and D7 in *T. spathiflorus* were the best performers hence the selection of parents could be based upon their performance. Taking internodal length as selection criterion for handicrafts, accessions D1 and D8 could be chosen. Similar selection of genotypes based on this character was done by Rasool (2011) in *Dendrocalamus strictus*.

The key leaf characters exhibited variations for size, texture and colour; but, the leaf characters area unit usually not enough to work out the species as indicated by Clark (1989). All genotypes were insignificantly completely different in leaf characteristics and bud breadth. Variability in terms of leaf length and leaf width was seen with best accessions D5 and D4 respectively in *T. spathiflorus*.

In the absence of flower or fruit characters, the culm sheath (Raizada and Chatterji 1963) and culm characters were treated as a pair of major categorization keys for the identification of bamboos (Das et al. 2007). The culm sheath is that the foremost important diagnostic feature for categorization and characterization of bamboos. Vegetative characters principally describing culm and culm sheath are widely used for bamboo species determination (Ohrnberger and Goerrings 1986) but, Wu (1962) expressed concern on the reliability of vegetative characters due to potential influence of atmosphere. Exclusively looking on culm and culm sheath variations, three taxonomic groups and a couple of varieties had been delineated in *T. spathiflorus* (Stapleton 1994). Six culm sheath features were taken for quantitative assessment of variation viz. culm sheath length. culm sheath breadth, blade length, total sheath length to breadth at base ratio, total length to blade length ratio and culm sheath area. Out of six culm characteristics; blade length and total sheath length/breadth at base were insignificant, however, culm sheath area, an important contributor of variability varied significantly. The variation in shape, size and colour of culm sheath of all the accessions of *T. spathiflorus* are clearly discernible (Fig. 4).

The high similarity among accessions in study represents less diversity that might be due to clonal nature. Clonal plant species tend to possess intermediate levels of genetic diversity (Ellstrand and Roose 1987) that is in keeping with the results obtained. Ten accessions of *T. spathiflorus* were grouped into two major clusters having six and four accessions, respectively.

The potential of RAPD technique to assess intra and interspecific genetic diversity of other bamboo species has al-

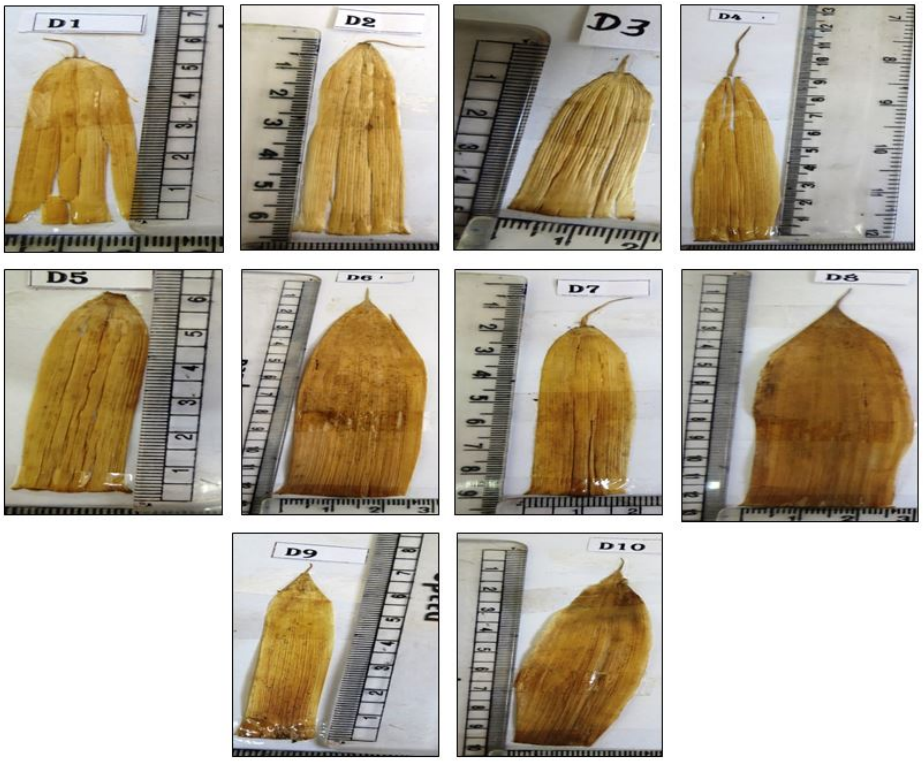

Fig. 4. Culm sheath variation among genotypes of *T. spathiflorus*.

ready been demonstrated earlier (Nayak et al. 2003). DNA profiling through RAPD technique has been used for the analysis of diversity and identification of duplicates within the large germplasm populations (Virk et al. 1995), phylogenetic relationship (Millan et al. 1996). Evidently, RAPD technology is a rapid and sensitive technique, which can be used to estimate relationships between closely and more distantly related species and groups of bamboo. RAPD profiles have been used often for developing phylogenetic relationship (Rout et al. 2003; Nanda et al. 2004; Tiwari et al. 2015). RAPD-RFLP is used as reproducible and informative method for screening differences among genera, species and varieties of bamboos (Konzen et al. 2017). A combination of both morphological and molecular studies refined the classical taxonomic studies (Doyle et al. 1994; Campbell et al. 1995), therefore an endeavor was made in the present study to make an account of variability study within genotypes of *T. spathiflorus* based on morphological as well as DNA profiling. Similar comparative study on characterization of *Thamnocalamus spathiflorus* subsp. spathiflorus at population was reported by Bhattacharya et al. (2009). Out of 42 primers screened, six RAPD primers viz. Primer-3, OPE08, OPF06, OPG12, OPN04 and OPN06 showed 100% polymorphism. Minimum polymorphism (70.00%) was recorded for OPAH03. Nayak et al. (2003) reported generation of 137 fragments from 12 bamboo species using 10 random primers with 100% polymorphism. The similar results were obtained in *T. falconeri* while using RAPD marker (Tiwari et al. 2015).The efficacy of selected primer for genetic diversity were obtained with very high average resolving power (Rp) value i.e. 12.78 per primer. Diversity on the basis of RAPD marker was remarkable between accessions pair because of their wide distribution pattern (Lalhruaituanga and Prasad 2009). The difference clearly provided edge of RAPD over morphology in diversity study of as the level of polymorphism was high with DNA based markers presumably because of no influence of environment on genotype prevailed.

Genetic variation and relationships based on cluster analysis of RAPD is being used immensely in bamboo classification and phylogeny. The potentiality of RAPD technique to assess intra and inter specific genetic diversity of other bamboo species has earlier been demonstrated (Nayak et

al. 2003). Present study reported higher morphological similarity than RAPD based assessment i.e. upto 93% among different accessions, may be due to the exposure of different genotypes in the same climatic conditions. Morphological appearance does not necessarily reflect the genetic (RAPD) status. This may be due to genotype environment interaction and the different combination of alleles/genes. These highly correlated characters might dominate the pattern of variation resulting in the distortion in cluster analysis. Cluster analysis based on jaccard's coefficient by RAPD markers given in Figs 1 and 2 show two major groups.

Conclusion

Present study reported higher morphological similarity than RAPD based assessment. The low variability (20 to 34%) resulted among the genotypes of *T. spathiflorus* may be due to deterioration of natural resources which in turn necessitates to adopt efficient conservation approaches in order to enrich the natural resources of the species. Unscientific harvesting should be discouraged and sustainability should be maintained. Proper extension activities should be assured to the local people for dissemination of knowledge about importance, scientific harvesting and sustainable as they play role in the success of any conservation program.

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References

Bhattacharya S, Ghosh JS, Das M, Pal A. 2009. Morphological and molecular characterization of Thamnocalamus spathiflorus subsp. spathiflorus at population level. Plant Syst Evol 282: 103.

Burley J, Styles BT. 1976. Tropical trees: variation, breeding, and conservation (Linnean Society symposium series; no. 2). Academic Press, London, pp 109-121.

Campbell CS, Donoghue JF, Baldwin BG, Wojciechowski MF. 1995. Phylogenetic relationships in Maloideae (Rosaceae): Evidence from sequences of the internal transcribed spacers of nuclear ribosomal DNA and its congruence with morphology. Am J Bot 82: 903-918.

Clark LG. 1989. Systematics of Chusquea Section Swallenochloa, Section Verticillatae, Section Serpentes, and Section Longifoliae (Poaceae-Bambusoideae). Syst Bot Monogr 27: 1-127.

Das M, Bhattacharya S, Basak J, Pal A. 2007. Phylogenetic relationships among the bamboo species as revealed by morphological characters and polymorphism analyses. Biol Plant 51: 667-672.

Doyle JA, Donoghue MJ, Zimmer EA. 1994. Integration of morphological and ribosomal RNA data on the origin of angiosperms. Ann Mo Bot Gard 81: 419-450.

Ellstrand NC, Roose ML. 1987. Patterns of genotypic diversity in clonal plant species. Am J Bot 74: 123-131.

Friar E, Kochert G. 1991. Bamboo germplasm screening with nuclear restriction fragment length polymorphisms. Theor Appl Genet 82: 697-703.

Friar E, Kochert G. 1994. A study of genetic variation and evolution of Phyllostachys (Bambusoideae: Poaceae) using nuclear restriction fragment length polymorphisms. Theor Appl Genet 89: 265-270.

Generoso AL, Santos JO, Carvalho VS, Sacoman NN, Rodrigues R. 2016. Proposal for qualitative and quantitative descriptors to characterise bamboo germplasm. Rev Ciênc Agron 47: 47-55.

Gunter LE, Tuskan GA, Wullschleger SD. 1996. Diversity among Populations of Switchgrass Based on RAPD Markers. Crop Sci 36: 1017-1022.

Huff DR, Peakall R, Smouse PE. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [Buchloë dactyloides (Nutt.) Engelm]. Theor Appl Genet 86: 927-934.

Kapur SK. 1990. Economically useful plants of majauri kirchi forest tract jammu and kashmir india ii verbenaceae to bambusaceae and gymnosperms. J Econ Taxon Bot 14: 523-534.

Kleinhenz V, Midmore DJ. 2001. Aspects of bamboo agronomy. Adv Agron 74: 99-153.

Kobayashi M. 1996. Phylogeny of world bamboos analysed by restriction fragment length polymorphisms of chloroplast DNA. In: International Symposium; London, UK; 25-29, March, 1996.

Kölliker R, Stadelmann FJ, Reidy B, Nösberger J. 1999. Genetic variability of forage grass cultivars: a comparison of Festuca pratensis Huds., Lolium perenne L., and Dactylis glomerata L. Euphytica 106: 261-270.

Konzen ER, Peron R, Ito MA, Brondani GE, Tsai SM. 2017. Molecular identification of bamboo genera and species based on RAPD-RFLP markers. Silva Fenn 51: 1691.

Kupicha FK. 1976. The infrageneric structure of vicia. R Bot Gard

Edinb 34: 287-326.

Lalhruaitluanga H, Prasad MNV. 2009. Comparative results of RAPD and ISSR markers for genetic diversity assessment in Melocanna baccifera Roxb. growing in Mizoram State of India. Afr J Biotechnol 8: 6053-6062.

Liu Z, Furnier GR. 1993. Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theor Appl Genet 87: 97-105.

Millan T, Osuna F, Cobos S, Torres AM, Cubero JI. 1996. Using RAPDs to study phylogenetic relationships in Rosa. Theor Appl Genet 92: 273-277.

Nair NV, Nair S, Sreenivasan TV, Mohan M. 1999. Analysis of genetic diversity and phylogeny in Saccharum and related genera using RAPD markers. Genet Resour Crop Evol 46: 73-79.

Naithani HB, Pal M, Lepcha STS. 2003. Gregarious flowering of Thamnocalamus spathiflorus and *T. falconeri*, Bamboos from Uttaranchal, India. Indian For 129: 517- 526.

Nanda RM, Nayak S, Rout GR. 2004. Studies on genetic relatedness of Acacia tree species using RAPD markers. Biologia 59: 115-120.

Nayak S, Rout GR, Das P. 2003. Evaluation of the genetic variability in bamboo using RAPD Markers. Plant Soil Environ 49: 24-28.

Ohrnberger D, Goerrings J. 1986. The bamboos of the world : a preliminary study of the names and distribution of the herbaceous and woody bamboos (Bambusoideae Nees v. Esenb.) documented in lists and maps. Ornberger, Odenthal, pp 187.

Prevost A, Wilkinson MJ. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98: 107-112.

Raizada MB, Chatterji RN. 1963. A new bamboo from South India. Indian For 89: 362-364.

Rasool S. 2011. Variability studies in *Dendrocalamus* strictus (Roxb.) Nees using protein fingerprinting and biochemical analysis. PhD thesis. Forest Research Institute (Deemed) University, Dehradun, India. pp 105-135.

Rout GR, Bhatacharya D, Nanda RM, Nayak S, Das P. 2003. Evaluation of genetic relationships in *Dalbergia* species using RAPD markers. Biodivers Conserv 12: 197-206.

Stange C, Prehn, D and Patricio A.J. 1998. Isolation of Pinus radiata Genomic DNA suitable for RAPD Analysis. Plant Molecular Biology Reporter, 16: 1-8.

Stapleton CMA. 1994. The bamboos of Nepal and Bhutan. Part I: Bambusa, Dendrocalamus, Melocanna, Cephalostachyum, Teinostachyum, and Pseudostachyum (Gramineae: Poaceae, Bambusoideae). Edinb J Bot 51: 1-32.

Tewari DN. 1992. A monograph on bamboo. International Book Distributors, Dehra Dun, 498 pp.

Tiwari C, Bakshi M, Nautiyal S. 2015. Morphological and Molecular Characterization of *Thamnocalamus falconeri* Hook f. ex. Munro. J For Environ Sci 31: 214-224.

Virk PS, Ford-Lloyd BV, Jackson MT, Newbury HJ. 1995. Use of RAPD for the study of diversity within plant germplasm collections. Heredity (Edinb) 74: 170-179.

Watanabe M, Ito M, Kurita S. 1994. Chloroplast DNA phylogeny of Asian Bamboos (Bambusoideae, Poaceae) and its systematic implication. J Plant Res 107: 253-261.

Wu MCY. 1962. The classification of Bambuseae based on leaf anatomy. Bot Bull Acad Sin 3: 83-108.

Zobel B, Talbert J. 1984. Applied forest tree improvement. John Wiley and Sons, New York, 505 pp.