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Morphological and Molecular Characterization of *Thamnocalamus falconeri* Hook f. ex. Munro

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Abstract

The economy of India and so also of many Asian countries depends on bamboos and their uses are not only in domestic items but also in rural housing and raw materials to several industries and germplasm characterization is an important link between the conservation and utilization of plant genetic resources. Classical taxonomic studies of the bamboos are based on floral morphology and growth habit, which can cause problems in identification due to erratic flowering coupled with different biotic agencies and environmental factors. Identification and genetic relationships among accessions of *Thamnocalamus falconeri* were investigated using morphology and random amplified polymorphic DNAs (RAPD) technique. Analysis started by using 51 vegetative characters and forty two 10-mer primers that allowed us to distinguish different genotypes hailing from different eco- zones of Garhwal Himalayas (India). The selected primers (12) were used for identification and for establishing a profiling system to estimate genetic diversity. A total of 79.33% polymorphism was estimated by using 12 selected primers. The genetic similar analysis was conducted based on binary digits i.e. presence (1) or absence (0) of bands, which revealed a wide range of variability among the species whereas genetic relatedness was quite high based on vegetative characters. Cluster analysis clearly showed two major clusters for both of the markers viz. morphology and RAPD belonging to 10 accessions of *T. falconeri*. Two major clusters were further divided into minor clusters. Cluster based on RAPD marker showed grouping of accessions of closed locality whereas analogy was reported for vegetative traits. The RAPD technique has the potential for use in species identification and genetic relationships studies of bamboo for breeding program.

Key Words: hill bamboo, genetic variability, polymorphism, RAPD analysis, *Thamnocalamus falconeri*

Introduction

Germplasm characterization is an important link between the conservation and utilization of plant genetic resources. Conservation and improvement of the plant genetic resources is the only way to fulfill human needs and greed and to preserve natural resources for future generations. Traditionally, morphological characters like growth habit, leaf type, floral morphology have been used to define taxa. Biotechnology can directly assist plant conservation programmes.

However, application of molecular techniques for the study of genetic diversity in bamboo has been limited. Studies include the use of (RFLP) Restriction Fragment Length Polymorphisms in *Phyllostachys* (Friar and Kochert 1991, 1994), chloroplast DNA Phylogeny of Asian bamboos (Watanbe et al. 1994) and world bamboos (Kobayashi 1997). Molecular DNA techniques allow researchers to identify genotypes at the taxonomic level, assess the relative diversity within and among the species and locate diverse

accessions for breeding purposes. Moreover, the commercial value associated with identifying useful traits creates a direct value on gene banks ensuring long-term preservation of a collection. RAPD assay is the cheapest and rapid method for identifying the genotypes within a short period and also requires only limited amount of DNA. The development of randomly amplified polymorphic DNA (RAPD) markers, generated by the polymerase chain reaction (PCR) using arbitrary primers, has provided a new tool for the detection of DNA polymorphism (Williams et al. 1990). RAPD analysis has been used to study genetic relationship in a number of grasses (Huff et al. 1993; Gunter et al. 1996; Kolliker et al. 1999; Nair et al. 1999).

Like other bamboo species, *T. falconeri* (Dev Ringal) also has erratic and long flowering cycles. Due to over usage of this resource and heavy extraction of the material from natural forests, the species is depleting at an alarming rate. The problem is further compounded by gregarious flowering resulting into death of entire clumps following seeding. The resultant regeneration takes time to establish and has to face intense grazing pressure, forest fires and upcoming weeds as the parent stock completely dries off after flowering. Responses recovered from the local people point to a heavy reduction in the population over the years. Many areas in hills where hill bamboo grew in dense thickets a decade ago now lie barren. The villagers/artisans are in short supply of this life supporting resource, threatening the livelihood of families, totally dependent on hill bamboos (locally known as Ringal).

In this context, 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.

Material and Methodology

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, 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

Comprehensive morphological study was conducted on score accessions based on various taxonomic descriptors. Each accession was considered as separate independent operational taxonomic unit (OTU). Twenty quanitative characters and thirty one qualitative characters were taken as morphological descriptors for each of the OTU's (3 replication per OTU) studied in the field. Mean values from three independent replications were used as OTU representative data for each of the quantitative morphological descriptors. The scored qualitative and quantitative data as cited in corresponding tables.

Genomic DNA isolation and RAPD study

Plant Materials

The young leaves of selected accessions (Table 1) were collected from Hill Bamboo Germplasm, Khirsu, Garhwal (India) (30° 10.368' N latitude, 78° 52.167' E longitude and 1,934 m altitudes above sea level), tagged properly in polybags and stored in ice bucket till they were brought to Plant Physiology Laboratory (FRI, Dehradun, India). The

Table 1. Geographical Details of different accessions of *Thamnocalamus falconeri* in Hill Bamboo Germplasm at Khirsu (India)

		T. falconeri
B1	13	FRH, Mandal I, Chamoli
B2	73	Van I, Bedni bugyal, Chamoli
B3	15	FRH II, Mandal, Chamoli
B4	77	Van II, Bedni bugyal, Chamoli
B5	103	Munsyari I, Pithoragarh
B6	16	Musk deer farm I Chopta, Chamoli
B7	80	Van III, Bedni bugyal, Chamoli
B8	17	Musk deer farm II Chopta, Chamoli
B9	12	Musk deer farm III Chopta, Chamoli
B10	100	Munsyari II, Pithoragarh

leaves were stored at -20°C in freezer (vest frost DFS 345) till use.

Plant DNA extraction

Total genomic DNA was extracted by modified method of Stange et al. (1998) from the leaves of ten selected accessions. Extracted genomic DNA sample was checked at 1% agarose gels and quantified using Biophotometer ($A_{260/280}$). Estimated purity of DNA sample was laid between 1.73-1.95 and DNA quantity ranged from 60.70-804.50 ng/μl (Table 2).

RAPD analysis

RAPD- PCR reactions was performed in 25 μl of re-action mixture containing10X Taq buffer, 2 mM dNTPs, 2.5 mM MgCl₂, Taq Polymerase (5 U/μl), 20 mM Primer and 50 ng/μl genomic DNA. The reaction was performed in a thermal cycler (Techne, FPROG05D) with a cycling program of intial denaturation 94°C for 4 min. followed by 94°C for 1 min., 37°C for 1min. and finally at 72°C for 2 min for 45 cycles. Final extention was done at 72°C for 5 min. The amplified (PCR) products were separated by elec-trophoresis on 1.5% (w/v) agarose gel with 1X TBE buffer stained with Ethidium bromide and photographed under Gel Documentation system (I.T. System UVP, Digi Doc).

Statistical Analysis

Morphological Analysis

One-way ANOVA (Analysis of variance) was performed by using Genstat version 3.2. The source of variation was accession. The F-value thus obtained was compared with the tabulated values at 0.1% level of significance and re-spective degrees of source and error. For better inter-pretation of significant results, critical difference (CD) or least significant difference (LSD) were calculated.

RAPD profile data

The amplified bands in the whole germplasm set was re-corded in a binary quantitative matrix as 1 (band present) and 0 (band absent). Reproducible amplified fragments of RAPD (bands present in each repetitions of each sample) were scored. 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 as described by (Prevost and Wilkinson 1999). The polymorphic information content (PIC) values for each of the amplified primers were estimated using the formula of (Lynch and Walsh 1998).

Cluster analysis

Morphology study comprised of fifty one morphological descriptors (18 cum sheath and 33 culm descriptors) were assessed for each of the ten OTU's. Mean values from three independent replications were used as OTU repre-sentative data for each of the quantitative morphological descriptors. The scored qualitative and quantitative data as cited in Tables 3 and 4 was standardized to construct a den-drogram via hierarchical clustering.

Jaccard's similarity index and UPGMA clustering were used to estimate RAPD variation. Each accession was con-sidered as a taxonomical operational unit (OTU) and RAPD data were transformed into a binary matrix and

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

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
B1	0.00									
B2	6.34	0.00								
B3	10.37	8.87	0.00							
B4	9.17	4.13	7.23	0.00						
B5	13.12	9.60	4.95	6.50	0.00					
B6	7.60	4.47	10.67	6.64	10.94	0.00				
B7	6.03	9.37	14.93	12.68	17.11	8.30	0.00			
B8	7.36	6.36	4.55	6.03	7.26	8.74	11.73	0.00		
B9	8.54	9.75	6.04	10.06	10.01	11.14	12.95	5.43	0.00	
B10	7.51	7.29	15.23	10.50	16.08	7.63	6.87	11.68	14.01	0.00

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

Accession	Number of nodes per Branch	Number of leaves per node	Bud Length (cm)	Bud Width (cm)	Culm Sheath Length (cm)	Culm Sheath Breadth (cm)	Blade Length (cm)	Total sheath length/Breadth at base	Total sheath length/Blade Length	Culm sheath area (cm^2)
B1	16.00	15.00	0.24	0.50	10.97	1.73	0.47	6.42	23.33	11.72
B2	15.67	17.67	0.29	0.54	13.73	1.33	1.33	10.49	11.12	15.82
B3	14.33	29.00	0.30	0.60	10.85	2.00	0.52	5.43	20.93	14.92
B4	10.00	7.00	0.41	0.60	11.80	3.17	1.67	3.74	8.11	33.02
B5	12.33	15.00	0.31	0.40	11.82	1.87	0.48	6.35	24.48	14.56
B6	16.67	26.33	0.20	0.50	17.57	2.00	0.73	8.78	25.60	22.85
B7	10.67	21.33	0.29	0.51	12.80	1.83	0.63	7.02	20.38	16.13
B8	12.67	11.67	0.50	0.64	9.96	2.43	0.23	4.13	44.90	16.32
B9	16.33	10.67	0.50	0.49	11.02	2.83	0.80	4.14	19.42	23.23
B10	12.67	13.33	0.31	0.38	13.80	2.77	0.30	5.02	49.61	27.33
Mean	13.73	16.70	0.33	0.52	12.43	2.20	0.72	6.15	24.79	19.59
Significance	NS	***	***	***	***	***	NS	***	***	***
C.D.	4.75	7.40	0.02	0.02	2.4	0.58	0.63	1.48	12.95	7.03

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)
B1	110.00	41.00	2.33	175.80	0.61	1.90	16.33	14.33	3.52	0.28
B2	153.00	30.00	7.00	144.40	0.44	4.37	12.87	33.00	2.45	0.26
B3	140.00	14.00	6.00	173.80	0.29	5.50	12.67	15.33	2.87	0.31
B4	165.00	30.00	4.00	183.90	0.45	3.33	13.93	25.00	2.69	0.32
B5	182.33	14.67	8.00	146.70	0.35	4.67	16.00	21.33	2.06	0.26
B6	120.67	24.00	6.00	152.80	0.34	1.73	15.50	14.33	2.91	0.44
B7	180.00	27.00	9.00	157.80	0.42	1.67	14.00	22.33	2.53	0.26
B8	84.00	40.33	5.33	174.30	0.48	3.67	10.23	31.00	2.60	0.26
B9	75.00	31.00	2.67	144.70	0.36	3.17	10.90	15.67	1.93	0.18
B10	115.00	15.00	2.33	136.80	0.29	2.33	12.10	18.00	3.33	0.27
Mean	132.50	26.70	5.27	159.10	0.40	3.23	13.45	21.03	2.69	0.28
Significance	***	***	***	***	NS	***	***	***	NS	NS
C.D.	11.95	4.16	1.73	21.66	0.22	1.51	2.36	3.32	0.94	0.25

***Significance at 0.1%.

Jaccard's similarity index was calculated. The Jaccard's distance matrices generated by RAPD using NTSYS-PC 2.11, dendrogram with bootstrap values was constructed by Darwin (version 5.0) software programme.

Results

Morphological Variability

The quantitative as well as qualitative data pertaining to morphological variations among accessions of *t. falconeri* are cited in Table 2.

Variation among different accessions of *T. falconeri* for clump circumference was quite significant. The accession with maximum clump circumference was B5 (182.33 cm)

from Munsyari I, Pithoragarh followed by B7 (180.00 cm) from Van III, Chamoli. Lowest was recorded in B9 (75.00 cm) from Musk deer farm III, Chopta. The accession displaying the highest average culm number was B1 (41.00) from FRH I, Mandal followed by B8 (40.33) from Musk deer farm II (Chopta). The minimum average number of culms was observed in accession B3 (14.00) from FRH II, Mandal. With respect to this character, accession B7 from Van III (Chamoli) recorded the maximum average number of young shoots (9.00), however minimum average number of young shoots (2.33) was recorded in accession B1 as well as B10 from FRH I, Mandal and Munsyari II (Pithoragarh) respectively. The accessions varied significantly with respect to the culm height. The accession B4

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

Characters	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
Clump density	1	2	0	1	0	2	1	3	3	2
Clump habit	3	1	1	1	1	3	3	3	3	3
Bending of culm	0	0	1	0	0	0	0	0	0	1
Culm colour	1	1	1	1	0	1	1	1	0	1
Culm surface	0	0	0	0	0	0	0	0	0	0
Leaf colour/dorsal	1	1	1	1	1	1	1	1	1	1
Leaf colour/ventral	0	0	0	0	0	0	0	0	0	0
Leaf habit	2	2	2	2	2	2	2	2	2	2
Leaf stalk	2	2	2	2	2	2	2	2	2	2
Leaf surface	1	1	1	1	1	1	1	1	1	1
Leaf texture	0	0	0	0	0	0	0	0	0	0
Internodal shape	0	0	0	0	0	0	0	0	0	0
Bud position	1	1	1	1	1	1	1	1	1	1
Bud shape	0	0	0	0	0	0	0	0	0	0
Bud number	1	1	1	1	1	1	1	1	1	1
Swollen node	1	1	1	1	1	1	1	1	1	1
Nodal sheath scar	1	1	1	1	1	1	1	1	1	1
Curved lower nodal branches	1	1	1	0	1	1	1	1	0	0
Piercing culm sheath	1	1	1	1	1	1	1	1	1	1
Ciliate margin	1	1	1	1	1	1	1	1	1	1
Pubescent adaxial hair	0	0	1	0	1	1	1	1	1	1
Pubescent abaxial hair	0	0	0	0	1	0	0	0	0	0
Blade reflexed	1	1	0	0	0	1	0	0	0	0
Hairy margin on blade	0	0	0	0	0	0	0	0	0	0
Ligule margin	0	0	0	0	0	0	0	0	0	0
Hairs on ligule	1	1	0	1	0	1	1	1	1	1
Bud arrangement	2	2	2	2	2	2	2	2	2	2
Auricle	1	1	1	0	1	1	1	1	0	1
Culm sheath texture	0	0	0	0	0	0	0	0	0	0
Culm sheath shape	2	2	2	2	2	2	2	2	2	2
Variable sheath Size	1	1	1	1	1	1	1	1	1	1

from Van II (Chamoli) had the maximum average culm height (183.90 cm) while minimum average culm length (136.80 cm) was observed in B10 from Munsyari II (Pithoragarh). Maximum of 16.33 cm length was recorded in accession B1 (FRH I, Mandal) at par with B5 (16.00 cm) while minimum (10.23 cm) in the accession B8 (Musk deer farm II, Chopta). Highest (33.00) internodes/culm were reported for accession B2 (Van I, Chamoli)) and minimum (14.33) in accessions B1 (FRH I, Mandal) as well as B6 (Musk deer farm I, Chopta). The maximum culm sheath area (33.02 cm^2) was discernible in B4 hailing from Van II Chamoli. Accession B1 from FRH I, Mandal exhibited the minimum sheath area (11.72 cm^2).

Cluster analysis

Dissimilarity matrix obtained using jaccard's coefficient revealed very high similarity with the range of similarity between 82.89-95.87%. The whole dendrogram was split into total two major clusters representing distribution of accessions of the localities of Mandal, Chopta, Van and Myunsari. Further, major clusters were divided into three sub- clusters which altogether formed seven minor clusters. Cluster I comprised of six accessions viz., B7, B1, B10, B4, B2 and B6 belong to Van III, FRH I (Mandal), Munsyari II, Van II, Van I and Musk deer farm I (Chopta) respectively. Cluster II comprised of four accession i.e. B8 (Musk

deer farm II, Chopta), B3 (FRH II, Mandal), B9 (Musk deer farm III, Chopta) and B5 (Myunsari I) as presented in Fig. 1. Accessions B2 and B4 represented maximum (95.87%) similarity and B5 and B7 showed minimum (82.89%) (Table 2).

RAPD Analysis

Initially, forty two RAPD primers were screened but only twelve were found polymorphic, hence they were kept for further analysis. A total of 103 bands were amplified in which 80 were polymorphic and 23 were monomorphic. Five RAPD primers viz. OPF04, OPQ06, OPP09, OPA19 and OPA17 were 100% polymorphic.

Least polymorphism was recorded for OPG12 (33.33). The resolving power (Rp) of the 12 RAPD primers used for the analysis ranged from 6.2 (OPQ06) to 19.0 (OPG12) with an average of 11.02 per primer. Polymorphism information content ranged from 0.13 (OPH05) to 0.41 (OPR07) with an average of value of 0.27 per primer (Table 5).

Cluster analysis

Data scored from 10 accessions of *T. falconeri* with 12 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 Fig. 2. At 63% similarity level, the dendrogram revealed two major clusters containing five accessions each which were further sub divided into sub-clusters and micro- clusters. The first major cluster included two sub- clusters and further categorized into three micro- clusters. First cluster was represented by five accessions viz. B1, B3, B6, B8 and B9 representing different localities on overall similarity of 70%. Second major cluster comprised of two sub- clusters and four micro- clusters having accessions B2, B4, B7, B5 and B10 at overall similarity of 68%. Similarity between different accessions was laid between 51 to 81%. Minimum similarity (51%) was found between accession pairs B1-B5 followed by B3-B5 (59%). Maximum of 81% similarity was found between accession pair B2-B4. Similarity of ac-
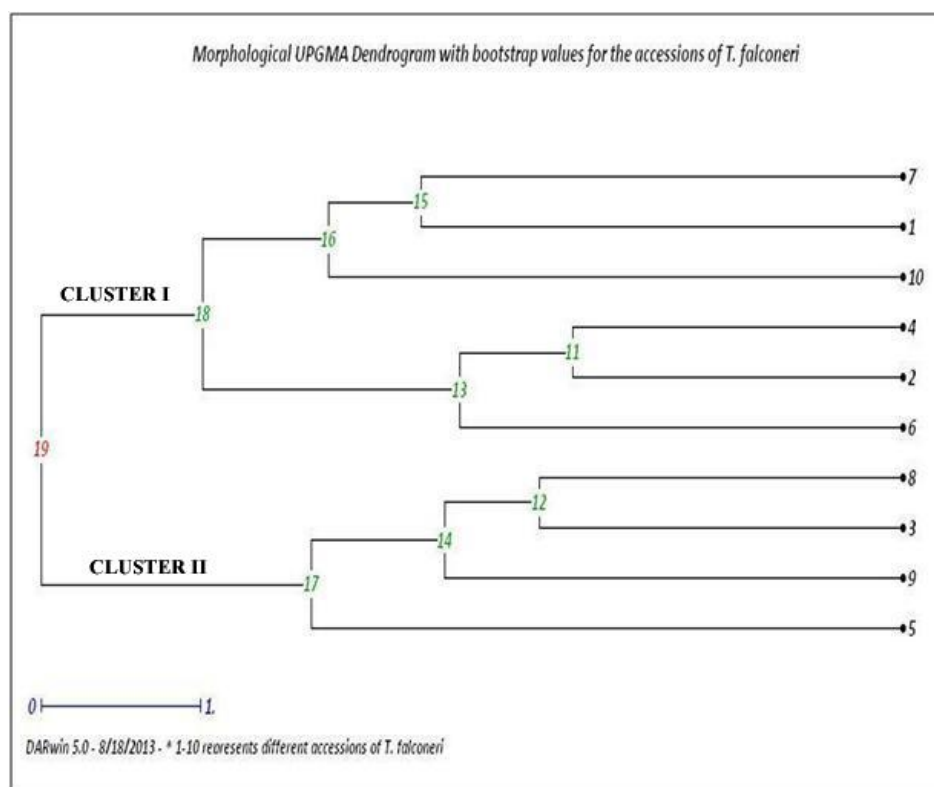

Fig. 1. Morphological UPGMA dendrogram with bootstrap values for the accessions of *T. falconeri*.

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

Primer	Primer seq. (5' − 3')	TNB	NPB	NMB	P %	Rp	PIC
OPF04	5'GGTGATCAGG3'	10	10	0	100.0	12.2	0.16
OPF06	5'GGGAATTCGG3'	9	8	1	88.88	11.0	0.21
OPG12	5'CAGCTCACGA3'	12	4	8	33.33	19.0	0.36
OPH05	5'AGTCGTCCCC3'	7	5	2	71.43	6.8	0.13
OPN06	5'GAGACGCACA3'	9	7	2	77.77	11.2	0.22
OPQ06	5'CCGTCGGTAG3'	5	5	0	100.0	6.2	0.29
OPP09	5'GTGGTCCGCA3'	10	10	0	100.0	9.4	0.32
OPR07	5'ACTGGCCTGA3'	9	5	4	55.56	14.0	0.41
OPR08	5'CCCGTTGCCT3'	8	5	3	62.50	12.6	0.20
OPA04	5'AATCGGGCTG3'	8	5	3	62.50	11.0	0.29
OPA19	5'TCTGTGCTGG3'	7	7	0	100.0	7.2	0.31
OPA17	5'GACCGCTTGT3'	9	9	0	100.0	11.6	0.37
Total		103	80	23	-	-	-
Average		8.58	6.67	1.92	79.33	11.02	0.27

*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.
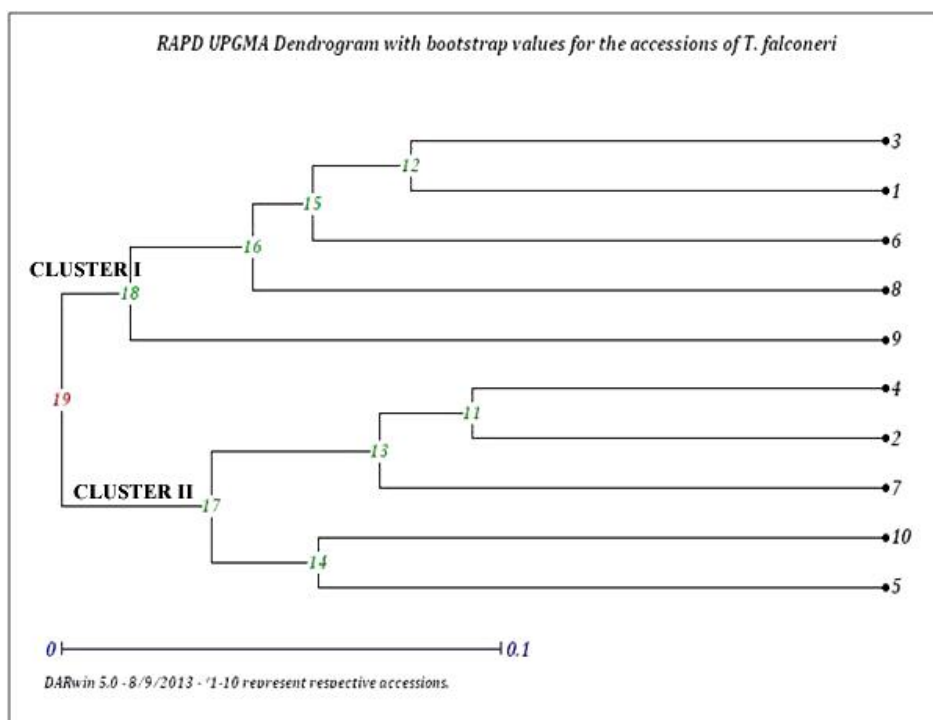

**Fig. 2.** RAPD UPGMA dendrogram with bootstrap values for the accessions of *T. falconeri*.

cession A10 with A5 and A6 was 48% and 59% respectively (Table 6).

Discussion

Due to long sexual cycles and unavailability of reproductive material in bamboos, vegetative characters (culm and

Table 6. Similarity matrix index is showing relatedness among the accessions of *T. falconeri* using RAPD marker
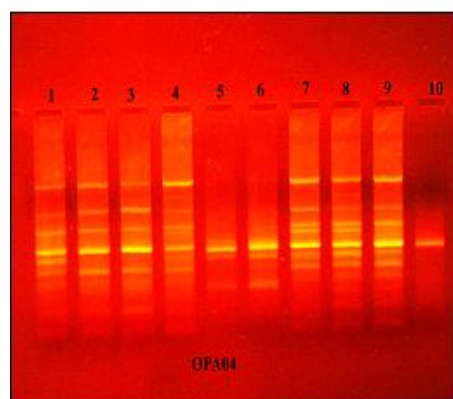
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
B1	1.00									
B2	0.68	1.00								
B3	0.78	0.71	1.00							
B4	0.61	0.81	0.62	1.00						
B5	0.51	0.71	0.54	0.72	1.00					
B6	0.72	0.65	0.76	0.58	0.62	1.00				
B7	0.63	0.78	0.65	0.76	0.69	0.65	1.00			
B8	0.69	0.65	0.72	0.59	0.57	0.72	0.71	1.00		
B9	0.68	0.62	0.63	0.58	0.62	0.65	0.63	0.67	1.00	
B10	0.62	0.66	0.59	0.70	0.74	0.63	0.68	0.63	0.71	1.00
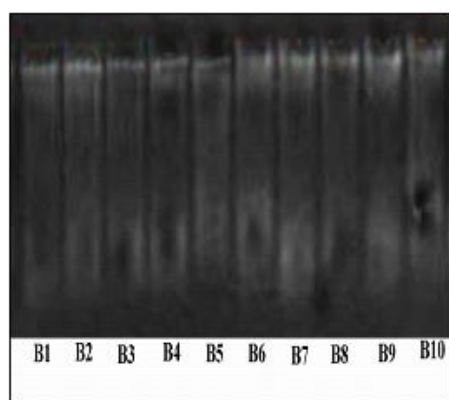
(a)
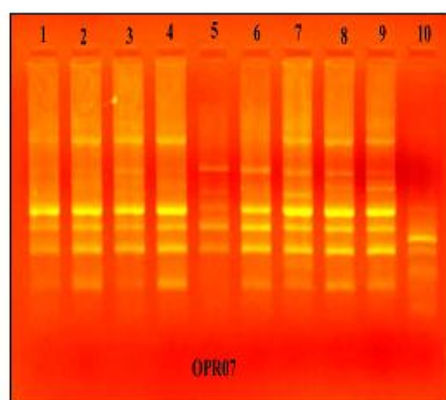
(b)

(c)

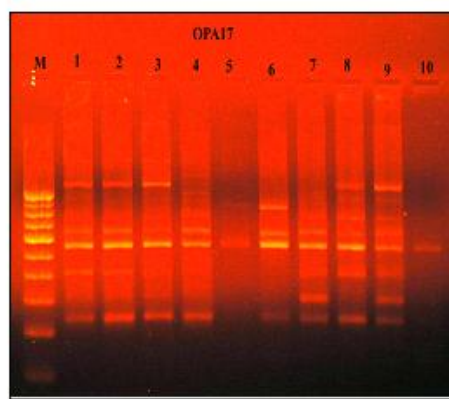**(d)**

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

culm sheath) are normally used as diagonostic tool for identification. The lack of remarkable morphological differences and erratic flowering make identification and delineation of genetic relationships in bamboos, specially in hill bamboos, very difficult. Moreover, smaller size and in-

distinguishing culm sheath makes characterization of different genotypes of hill bamboo species more difficult. Phylogenetic studies using vegetative characters have been quite defeating as they are affected by environmental factors (Wu 1962) and hence, are less reliable for systematic stud-

Fig. 4. Culm sheath variation among accessions of *T. falconeri*.

ies (Ohrenberger 2002) and phylogenetics. Because of several limitations of morphological data, traditional method of identifying species by morphological characters are gradually substituted or supplemented by DNA profiling. Several authors (Tiwari 1992; Bedell 1997; Kumar and Stephen 1999) emphasized the need for more compre-

hensive description of bamboos for identification, effective utilization and conservation through multidisciplinary approach. The DNA markers generated by RAPDs have been proven to be useful for studying genetics and phylogenetic relationships in a wide range of perennial plants. The potentiality of RAPD technique to assess intra and inter

specific genetic diversity of other bamboo species has already been demonstrated earlier (Hsiao and Rieseberg 1994; 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).

To resolve the discrepancy that might arise when taking morphological manifestations alone, RAPD/ biochemical markers combined with morphological characters could validate the phylogenetic relationships among bamboo taxa, hence molecular evidences need to be supported by morphological or biochemical data. A combination of both morphological and molecular studies refined the classical taxonomic studies (Doyle et al. 1994; Campbell et al. 1995; Endress et al. 1996), therefore an endeavor was made in the present study to make an account of variability study within genotypes of *T. falconeri* 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. in 2009.

Analyses of variance revealed significant variation in culm height of *T. falconeri* genotypes which showed gross agreement of the result reported in *T. spathiflorus* by Bhattacharya et al. (2009) but other vegetative characters viz. culm diameter, total sheath length to breadth at base ratio and total sheath length to blade length ratio failed to impart significant variation. Substantial variation was observed in majority of traits which are exploitable for future bamboo breeding programmes. Among all ten genotypes, Accession B1 from FRH, Mandal I, Chamoli, Uttarkashi was the best genotype displaying best average growth among accessions of *T. falconeri*.

The primer screening step in 42 decamer primers revealed GC of 60 to 70 %. Out of 42, only 12 high polymorphic primers were retained for RAPD analysis with an average polymorphism value (%) of 79.33. Nayak et al. (2003) reported generation of 137 fragments from 12 bamboo species using 10 random primers with 100%

polymorphism. The range of PIC value of primers was 0.16 to 0.41. Primers viz. OPR-07, OPA 17 and OPG 12 were most efficient primer with highest PIC 0.41, 0.37 and 0.36 respectively.

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. Higher estimates of genetic diversity with RAPD markers over other marker such as isozymes have been reported earlier (Liu and Furnier 1993; Kongkiatngam et al. 1995). Present study reported higher morphological similarity than RAPD based assessment i.e. upto 90% 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 Fig. 1 & 2 show two major groups. The accessions collected from different locations of Chopta District were found in the same clusters groups whereas an analogy was observed in the grouping pattern based on morphological traits.

Results and findings of the study revealed low variability which is reflectance of deterioration of natural resources of *T. falconeri*. Therefore, there is a need to develop different conservation approaches and enrichment of natural resources of the species. Moreover, unscientific harvesting should be kept under control. Since, local communities have most important role in the success of any conservation program, dissemination of knowledge about importance, scientific harvesting and sustainable uses of Ringal among local communities through various extension programmes is the need of hour.

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References

Bedell PE. 1997. Taxonomy of bamboos. APC publications, New Delhi, 150 pp.

Bhattacharaya S, Ghosh JS, Das Malay, Pal A. 2009. Morphological and molecular characterization in *Thamnocalamus spathiflorus.* subsps. *spathiflorus* at population level. Plant Systematics and Evolution 282: 13-20.

Campbell CS, Donoghue MJ, 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. American Journal of Botany 82: 903-918.

Doyle JA, Donoghue MJ, Zimmer EA. 1994. Integration of morphological and ribosomal RNA data on the origin of the angiosperms. Annals of the Missouri Botanical Garden 81: 419-450.

Endress ME, Sennblad B, Nilsson S, Civeyrel L, Chase MW, Huysmans S, Grafstr E, Bremer B. 1996. A phylogenetic analysis of Apocynaceae s.str. and some related taxa in *Gentianales*: a multidisciplinary approach. Opera Bot Belg 7: 59-102.

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.

Gunter LE, Tuskan GA, Wullschleger SD. 1996. Diversity among populations of switchgrass based on RAPD markers. Crop Science 36: 1017-1022.

Hsiao JY, Rieseberg LH. 1994. Population genetic structure of *Yushania niitakayamensis* (Bambusoideae, *Poaceae*) in Taiwan. Molecular Ecology 3: 201-208.

Huff DR, Peakall R, Smouse PE. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. Theoretical and Applied Genetics 86: 927-934.

Kobayashi M, Chapman GP. 1996. Phylogeny of world bamboos analysed by fragment length polymorphism of chloroplast DNA. The Bamboos. Proceeding of an International Symposium, London 25-29: 227-236.

Kolliker R, Stadelmann FJ, Reidy B, Nosberger 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.

Kongkiatngam P, Waterway MJ, Fortin MG, Coulman BE. 1995. Genetic variation within and between two cultivars of red clover (*Trifolium pratense* L.): Comparisons of morphological, isozyme and RAPD markers. Euphytica 84: 237-246.

Kumar M, Stephen S. 1999. Concepts in bamboo taxonomy--past, present and future: a global perspective. In: Biodiversity taxonomy and conservation of flowering plants (Sivadasan M, Mathew P, eds). Mentor Books, Calicut, 396 pp.

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. African Journal of Biotechnology 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. Theoretical and Applied Genetics 87: 97-105.

Lynch P, Walsh B. 1998. Genetics and analysis of quantitative traits. Sinauer Assocs. Inc., Sunderland, MA, 980 pp.

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. Genetic Resources and Crop Evolution 46: 73-79.

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 bamboousing RAPD markers. Plant Soil Environ 49: 24-28.

Ohrnberger D. 2002. The bamboos of the world: annotated nomenclature and literature of the species and the higher and lower taxa. Elsevier Science, Amsterdam, 112 pp.

Prevost A, Wilkinson MJ. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics 98: 107-112.

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

Tiwari DN. 1992. A monograph on Bamboo. Inter-national Book Distributors, Dehra Dun, India, 498 pp.

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

Watanabe M, Ito M, Kurita S. 1994. Chloroplast DNA phylogeny of Asian bamboos (*Bambusoideae, Poaceae*) and its systematic implication. Journal of Plant Research 107: 253-261.

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531-6535.

Wu MCY. 1962. The classification of Bambuseae based on leaf anatomy. Bot Bull Academia Sinica 3: 83-107.