Evaluation of Genetic Diversity in Central Indian Bamboo provenances using RAPD markers

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Abstract: Bamboo is one of the important plant for pulp, paper and charcoal industries. After China, India is the second largest bamboo reserve in Asia. Around the globe, wide genetic diversity of bamboo is present which serves as the base for selection and improvement. DNA based molecular markers appears to be powerful techniques for characterizing and evaluating genetic diversity in 33 genotype of bamboo from central India. Out of ninety primers, seventy four primers gave poor or no amplification at all, while sixteen primers produced distinct, highly reproducible amplified polymorphic products for all the 33 provenances. The RAPD primers produced 976 DNA fragments across 33 provenances, of which 961 were polymorphic. The number of amplified bands varied from 36 to 103, which varied in size from 100 to 1050 bp. The percentage polymorphism ranged from 93.62 to 100 with an average percentage polymorphism of 98.46. The PIC value varied from 0.348 to 0.719. The Jaccard's similarity coefficient ranging from 0.25 to 1.00. The dendrogram revealed that thirty three genotype collected from different location of central India classified in to two major cluster A and B showed 29 percent similarity.

Key words: Dendrocalamus, strictus, RAPD, Molecular marker, Genetic diversity, Dendrogram

Introduction

India has the second largest reserve of bamboo populations in the world. The fibres of bamboo are mainly used in the pulp, paper and charcoal industries, while the culms have several other uses as 'poor man's timber'. The total annual bamboo demand in India has been estimated to be approx. 5 million tonnes, of which about 3-5 million tonnes are required by the paper and pulp industry alone (Sharma, 1987). *Dendrocalamus strictus* is one of the strongest species and is thus preferred for paper pulp production; it is also used for construction purposes (Bhatt *et al*., 2003). The species is suitable for the production of quality paper due to its long fibres (Upreti and Sundriyal, 2001) and also for producing furniture. Among the anatomical characteristics of bamboo culm, fibre length is important for technical evaluation, especially in the pulp and fibre-based industries. Fibre wall thickness of the culm predetermines the pulping characteristics, paper quality, permeability and strength relationships (Mohmod, 2001). *Bambusa balcooa* and *Bambusa. tulda* are two abundant tropical species that are recognized as priority bamboo species by the FAO amongst eighteen other bamboo species found globally. Due to the unusually long sexual cycle and unavailability of any other diagnostic tool, identification of bamboo is mainly dependent on vegetative descriptors such as culm morphology, and the morphology of the culm-sheath including ligule and auricle (Ohrnberger and Goerrings, 1986).

Recent advances in molecular biology, principally the development of PCR, have provided powerful techniques for characterizing and evaluating genetic diversity and population evolution. RAPD (Williams *et al.,* 1990) and ISSR markers (Zietkiewicz *et al*., 1994) are two widely applicable techniques to identify relationships at the species and cultivar levels (*Raina et al*., 2001; Martins *et al*., 2003; Gupta *et al*., 2008; Arif *et al*., 2009), because they are rapid, simple to perform and inexpensive; they do not require prior knowledge of DNA sequences and only a small amount of DNA is needed (Esselman *et al*., 1999). Genetic diversity some of bamboo species has been evaluated with molecular techniques; however, genetic studies of rare and valuable in central Indian bamboo provenances (*Dendrocalamus* species), have not been reported. Thus investigation was carried out to elucidate the information on genetic variation among 33 provenances of *D. strictus* using RAPD markers.

Materials And Methods

The clones of different provenances from central India (Maharashtra, Madhya Pradesh and Chhattisgarh) in natural stand were grown in a nursery bed (Table 1). Total DNA was extracted from fully expanded leaves of 33 clones of *D. Strictus* provenances collected from individual clone in nursery**.**

Table 1. Principal geographical features of the collection sites (*Dendrocalamus strictus*)

Sl.No	Location	Zone/ Province	Topography	Stand	Wind condition	Grazing intensity
01.	Gondia	Gondia (MH)	Gentle slope	Natural	Intermediate	Moderate
02.	Melghat	Amravati (MH)	Hilly	Natural	Intermediate	Moderate
03.	Gutta	Gadchiroli (MH)	Hilly	Natural	Intermediate	Moderate
04.	Ballarshah	Chandrapur (MH)	Gentle slope	Natural	Intermediate	Moderate
05.	Tadoba	Moharli (MH)	Gentle slope	Natural	Intermediate	Moderate
06.	Chichpalli	Chandrapur (MH)	Gentle slope	Natural	Intermediate	Moderate
07.	Allapalli	Allapalli (MH)	Medium slope	Natural	Intermediate	Moderate
08.	Bedgaon	Kurchi (MH)	Hilly	Natural	Intermediate	Moderate
09.	Gadchiroli	Gadchiroli (MH)	Gentle slope	Natural	Intermediate	Moderate
10.	Bhamragarh	Bhamragarh (MH)	Hilly	Natural	Intermediate	Moderate
11.	Wardha	Wardha (MH)	Flat	Natural	Intermediate	Moderate
12.	Ashti	Ashti (MH)	Flat	Natural	Exposed	Light
13.	Chindwara	Chindwada (MP)	Gentle slope	Natural	Exposed	Moderate
14.	Langi	Langi (MP)	Gentle slope	Natural	Exposed	Moderate
15.	Lamta	Balaghat (MP)	Hilly	Natural	Exposed	Moderate
16.	Ukawa	Balaghat (MP)	Hilly	Natural	Intermediate	Moderate
17.	Betul	Betul (MP)	Gentle slope	Natural	Exposed	Moderate
18.	Jabalpur	Jabalpur (MP)	Medium slope	Natural	Intermediate	Moderate
19.	Katni	Katni (MP)	Medium slope	Natural	Exposed	Light
20	Mandla	Mandla (MP)	Hilly	Natural	Intermediate	Moderate
21.	Kanha	Kanha (MP)	Gentle slope	Natural	Intermediate	Moderate
22.	Shahdol	Shahdol (MP)	Gentle slope	Natural	Intermediate	Moderate
23.	Surajpur	Surajpur (CG)	Hilly	Natural	Intermediate	Moderate
24.	Bilaspur	Bilaspur (CG)	Medium slope	Natural	Intermediate	Moderate
25.	Achanakmar	Bilaspur (CG)	Hilly	Natural	Intermediate	Moderate
26.	Ambikapur	Ambikapur (CG)	Gentle slope	Natural	Intermediate	Moderate
27.	Katghora	(CG)	Medium slope	Natural	Intermediate	Moderate
28.	Kondagaon	(CG)	Hilly	Natural	Intermediate	Moderate
29.	Rajnandgaon	Rajnandgaon (CG)	Hilly	Natural	Intermediate	Moderate
30.	Baikunthpur	(CG)	Hilly	Natural	Intermediate	Moderate
31.	Manendragarh	Manendragarh (CG)	Flat	Natural	Intermediate	Moderate
32.	Raigarh	Raigarh (CG)	Flat	Natural	Exposed	Light
33.	Bharatpur	Bharatpur (CG)	Gentle slope	Natural	Exposed	Moderate

Isolation of genomic DNA

The genomic DNA of bamboo (*Dendrocalamus strictus)* from 33 clones of young leaves was extracted by using Cetyl Trimethyl Ammonium Bromide (CTAB) method described by Doyle and Doyle (1990) with few modifications (viz., amount of leaf tissue and temperature). Fresh leaf tissue (0.1 g) were crushed in liquid nitrogen and transferred into a 2 ml pre-sterilised centrifuge tube. 0.5 ml of extracted buffer [100 mM tris-HCl, 0.5 Methylenediaminetetraacetic acid (EDTA), 3.5 M NaCl, 3% cetyltrimethyl ammonium bromide (CTAB), 1& polyvinylpyrrolidone and 0.2 M β -mercaptoethanol pH 8.0] was added in crushed sample and incubated at 65^0C for 60 min. after incubation sample were emulsified with an equal volume of chloroform, isoamyl alcohol (24:1) and

supernatants were transferred into new pre-sterilized centrifuge tube and it was repeated twice. Precipitation of DNA was carried out using the mixture of chilled absolute alcohol and 3 M sodium acetate and by overnight incubation at - 20^0 C finally DNA was precipitated by chilled 80% ethanol. The precipitated DNA was air dried and dissolved in 100 μl of Tris-EDTA (TE) buffer. Total pure DNA was quantified by Nanodrop 1000 spectrophotometer. DNA of all 33 clones were diluted to 20 ng / μl with nuclease free water and kept at 4 ºC.

RAPD analysis was carried out following Williams *et al.* (1990). The reaction was carried out in a 20 μL reaction cocktail consisting of 20 ng template DNA, 10X Taq polymerase Buffer (10 mM Tris-Hcl pH 8.0, 50 mM KCl, 0.1%w/v Trition X 100), 3.2 mM

MgCl$_2$, 0.4 µM Primer, 250 µM of each dNTP and 1U Taq DNA Polymerase (Qiagen). The PCR reaction was carried out in a thermocycler programmed for an initial denaturation of 94^{0}C for 4 minutes followed by 35 cycles of denaturation at 94^{0}C for 1 minute, primer annealing at 55^{0}C for 1 minute, extension at 72^{0}C for 2 minutes and final extension at 72^{0}C for 10 minutes. The amplified products were electrophoresed on 1.8% agarose gel run in 1X TAE buffer at constant current of 120 V and documented using Gel Documentation system.

Screening of specific RAPD amplicon

Ninety Random primers (OPA 1-15, OPD 1-10, OPB 1-10, OPG 1-10, OPY 5-15, OPE 1-5, OPB 10-15, OPM 10-20, RPi-C 1-10 and HB 10-20) were screened by RAPD for estimating the genetic diversity.

The screening of primers resulted in 16 RAPD primers which showed polymorphisms with all 33 provenances.

The image profiles of banding patterns were recorded and molecular weight of each band was determined by running 1 kb ladder along with the amplicons. The banding pattern was scored based on the presence or absence of clear, visible and reproducible bands (1 for present and 0 for absent). The binary data (1/0) was used to generate a similarity coefficient. Genetic similarity(S), between provenances was estimated by using Jaccard's coefficient. Pairwise comparisons based on the similarity matrix generated by this analysis were used to generate dendrograms of genetic relatedness by Unweighted pair group method with airthematic averages (UPGMA) using NTSYS-pc (Numerical taxonomy system, applied bio-statistics, Inc., New York, USA, software version 2.02e)

Table 2: Nucleotide sequence used for study of genetic variation of 33 provenances of *D strictus*

S. No.	RAPD Primers	Sequence of primer	S. No.	RAPD Primers	Sequence of primer
1	OPA12	TCGGCGATAG	9	OPM16	GTAACCAGCC
2	OPD1	ACCGCGAAGG	10	OPM18	CACCATCCGT
3	OPB4	GGACTGGAGT	11	RPi-C1	AAAGCTGCGG
4	OPG4	AGCGTGTCTG	12	RPi-C2	AACGCGTCGG
5	OPY11	AGACGATGGG	13	RPi-C5	AATCGGGCTG
6	OPE1	CCCAAGGTCC	14	RPi-C6	ACACACGCTG
7	OPB11	GTAGACCCGT	15	HB10	GAGAGAGAGAGACC
8	OPD5	TGAGCGGACA	16	HB12	CTGTGTGTGTGTGTGTCC

Result And Discussion

Genomic DNA was isolated from fresh leaf tissues of all the 33 provenances of *D. strictus* collected from different locations (Table 1). Total ninety primers used for the initial screening for polymorphism with 33 provenances of *Dendrocalamus strictus*. Out of ninety primers, seventy four primers gave poor or no amplification at all while sixteen primers (Table 2) produced distinct, highly reproducible amplified polymorphic products for all the provenances (Figure 1 and 2). These 16 primers were then used for RAPD analysis, produced 976 DNA fragments that could be scored in all provenances. The number of amplified fragment varied from 36 (RPi-C6) to 103 (HB12), which varied in size from 100 to 2500 bp. Of the 976 amplified bands, 961 were polymorphic, with an average of 60.063 polymorphic bands per primer. Percent polymorphism ranged from 93.62 (OPB11) to 100 (OPG4, OPD5, RPi-C1 and HB10), with an average percentage polymorphism of 98.46**.** The PIC value varied from 0.348 (RPi-C6) to 0.719 (HB12).

Figure 1 and 2 shows a representation of the extensive polymorphism observed among the *D. strictus* provenances, as revealed by RAPD primer HB10 and HB12. A dendrogram generated based on UPGMA algorithm by using NTSYS-pc software for RAPD molecular data. The dendrogram grouped the 33 provenances into three main clusters (Fig. 3.) with Jaccard's similarity coefficient ranging from 0.25 to 1.00. The dendrogram revealed that thirty three provenances collected from different location of central India classified in to two major cluster A and B. Only two provenances namely DSCP9 (Gadchiroli) and DSCP33 (Bharatpur) showed as out group with 75 percent dissimilarity.
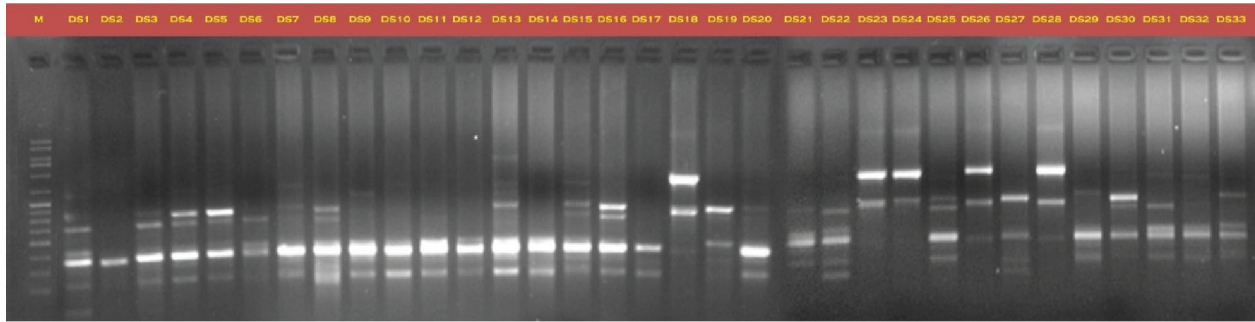
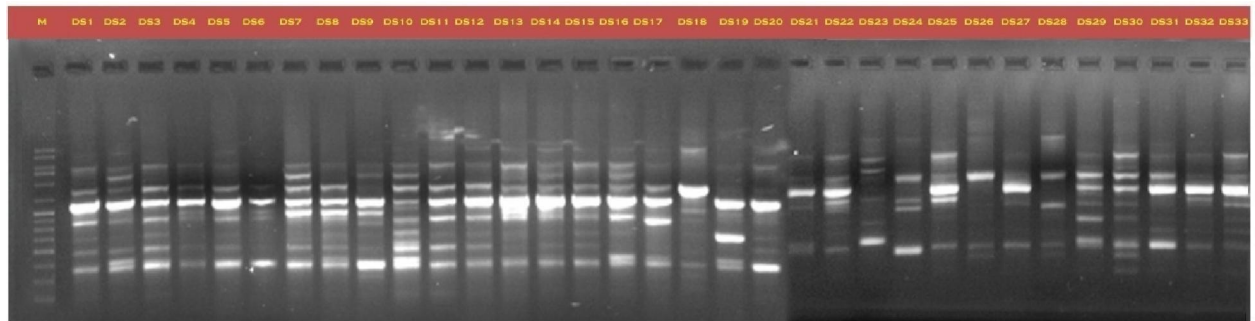**Fig 1: RAPD pattern of a 1-33 provenances of *D. strictus* using primer HB10**

Fig 2: RAPD pattern of a 1-33 provenances of *D. strictus* using primer HB12

Table 3. Table show total amplified fragments, polymorphic bands, % polymorphism and PIC in 33 provenances of *D. strictus*

S. No.	RAPD Primers	Amplified product (bp)	Poly band	Mono band	Total bands	% polymorphism	PIC
1	OPA12	200 -800	77	1	78	98.72	0.499
2	OPD1	100 -650	63	1	64	98.44	0.481
3	OPB4	200 -700	58	1	59	98.31	0.459
4	OPG4	200 -1000	72	0	72	100.00	0.496
5	OPY11	200 -800	54	1	55	98.18	0.444
6	OPE1	400 -1000	63	1	64	98.44	0.475
7	OPB11	200 -900	44	3	47	93.62	0.407
8	OPD5	100 -500	49	0	49	100.00	0.418
9	OPM16	200 -850	42	1	43	97.67	0.385
10	OPM18	400 -900	56	1	57	98.25	0.491
11	RPi-C1	200 -600	36	0	36	100.00	0.397
12	RPi-C2	200 -800	64	2	66	96.97	0.480
13	RPi-C5	100 -600	72	1	73	98.63	0.494
14	RPi-C6	100 -400	36	1	37	97.30	0.348
15	HB10	100 -1400	73	0	73	100.00	0.493
16	HB12	250 -2500	102	1	103	99.03	0.719
Total Mean			961 60.063	15 0.938	976 61.000	1573.56 98.348	7.486 0.468

Cluster A and cluster B showed around 29 percent similarity to each other. In cluster A 16 bamboo provenances were included, while in cluster B 15 provenances included. Cluster A is further classified into two sub cluster A1 and A2 with 36 per cent dissimilarity to each other. Sub cluster A1 included ten provenances while sub cluster A2 included only six provenances. Maximum similarity in sub cluster A1 was observed between DSCP10 (Bhamragardh) and DSCP12 (Ashti) is 86 percent, while in sub cluster A2 it was observed in DSCP6 (Chichpalli) and DSCP14 (Langi) which was 79 percent similarity (Table 3).

The cluster B is also classified into two sub cluster B1 and B2. Sub cluster B1 consisted 11 provenances, while four provenances were gouped in sub cluster B2. In sub cluster B1 maximum similarity was observed between DSCP24, DSCP26 and DSCP28

and that was 100 percent, while in sub cluster B2 maximum similarity evidenced 57 percent between DSCP13 and DSCP20. DSCP9 (Gadchiroli, MH) and DSCP33 (Bharatpur, CG) showed 75 percent dissimilarity with rest of the provenances.

The genetic structure of a species is affected by a number of evolutionary factors including mating system, gene flow and mode of reproduction, as well as natural selection (Hamrick *et al*., 1992). The results showed that the genetic diversity of this samples is higher, possibly due to large distance between the populations in the present study, varying from 150 to 1750 km. The genetic differentiation of provenances of *D. strictus* could broadly be explained as a results of aboitic (geographical e.g.- hydrographic connection or climatic differentiation) factors.
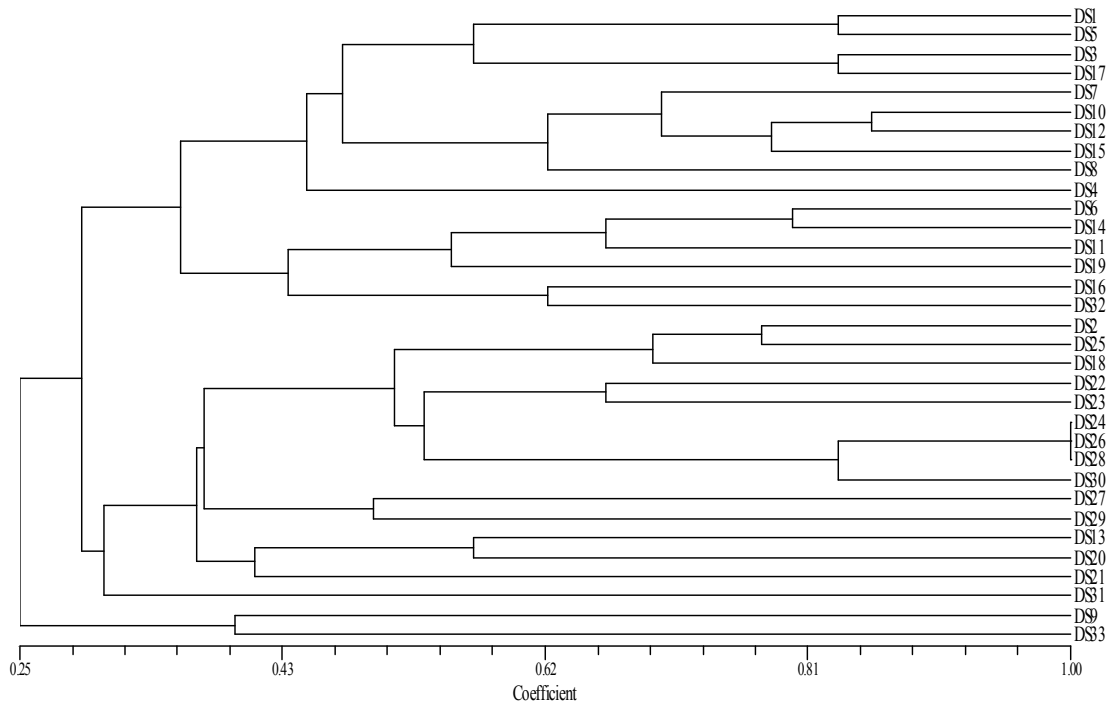

Fig.**3**- **UPGMA dendrogram of cluster analysis of RAPD markers illustrating the genetic relationship among the 33 provenances of *D. strictus***

Several reports are available to demonstrate the use of RAPD markers for determination of genetic variation in plants. Jain *et al*., (2003) studied molecular diversity in *Phyllanthus amarus* by RAPD profiling of 33 collections from different location using MAP primers. Mathur *et al*., (2008) studied genetic fidelity of micro-cloned progeny of *Chlorophytum borivilianum*. They scored 79 amplified reproducible monomorphic bands with three different

sets of 24 decamer primers (14 MAP, 3 OPO, 7 OPA primers). Khanuja *et al*., (2000) used 60 random primers to analyze 11 accessions from six taxa of Mentha. Nanda *et al.,* (2004) used forty primers and selected 17 primers on the basis of their ability to detect distinct, clearly resolved and polymorphic amplified product for the analysis of six species of Acacia. They found high degree of diversity (70%) within the six tree species of Acacia. Abd-El-Haleem

et al., (2009) reported genetic analysis and RAPD polymorphism in Wheat Provenances. Naugzemys *et al*., (2007) reported genetic variation and relationship among 39 accessions of *Lonicera caerulea* and one accession of *L. xylosteum*. Batitini *et al.*, (2009) evaluated the genetic diversity of seven populations of *Anemopaegma arvense,* using random amplified polymorphic DNA markers. Ponnuswami *et al*., (2008) used RAPD markers for identify the desirable traits in Palmyrah palm. Maia *et al*., (2009) used random amplified polymorphic DNA (RAPD) markers to detect polymorphism and to examine relationships among four table grape clones from NorthWestern Paraná, in Southern Brazil. Khurana *et al*., (2012) evaluated the genetic variation in the *Jatropa curcas* using RAPD and ISSR marker. Suwanchaikasem *et al*., (2012) demonstrated that *T. laurifolia* was successfully distinguished from its related species based on their molecular signatures. They show that RAPD analysis as a technique that is able to examine the phylogenetic relationship of different plant species. Osman *et al*., (2012) used RAPD marker for the identify Eucalyptus species genome. However, the present study indicates that the RAPD technique is a useful tool for the identification of germplasm analysis and genetic relationships between and within the *D. strictus* genetic resources. The relatively large number of polymorphisms obtained seems due to large Phylogenetic distance among these taxa. It would allow a more quantitative assessment of genetic distances between species. Such an analysis, together with data from other classical methods, could thus be used to make a more accurate reconstruction of the *D. strictus* genetic resources evolution. Furthermore, such an approach might be helpful in identifying taxa of potential value in genetic improvement programmes.

Conclusion

The present study is the first report that provides genetic relationship among the various provenances of *D. strictus* from central India. The majority of RAPD variations shown large genetic diversity present between different provenances of *D. Strictus* populations. It is suggested that RAPD markers could be successfully applied for detecting genetic variability in natural population of *D. strictus*. Moreover RAPD marker will have a major impact on the conservation and improvement of species *D. strictus*.

Acknowledgement

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