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MOLECULAR TAXONOMIC STUDY OF ELEVEN BAMBOO TAXA FROM ASSAM AND NORTH EAST INDIA BASED ON RAPD PROFILE

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ABSTRACT

The present study attempt to ascertain genetic diversity and phylogenetic relationship among eleven taxa of bamboo from NE India, particularly Assam. Five primers were used in the present study. Randomly amplified DNA profile by RP1-10 revealed a total 28 DNA amplicons in the size range of 120 bp to 1800. All the taxa exhibited diverse DNA profile indicating wide genetic diversity. S1 matrix shows that out of 55 pairwise combinations only 5.4% showed S1 value little above 50.0%. Dendogram analysis revealed two major clusters with wide phylogenetic distances among the taxa. Among the taxa, *Dendrocalamus giganteus* appear to be most distantly related to rest. On the other hand *D. hamiltonii* (D) and *D. hamiltonii* (H) which are considered as same species from conventional taxonomic view appear to be two different species with SI value of 26.8%.

Key words: Bamboo; RAPD, Molecular taxonomy, Dendrogram

INTRODUCTION

Understanding taxonomical status of a species and genetic diversity is of paramount importance not only for academic purpose but also for genetic improvement programmes, sustainable use and conservation. Among all economically important plant species bamboo

taxonomy is most problematic and a perpetual riddle. This is because conventional taxonomy is mainly based on flower characteristics and morphological features. But bamboo flowering is rare and highly irregular. Species like *Melacanna baccifera* have a flowering cycle of 45 years; others like *Dendocalamus strictus* have irregular cycle of 30 to 70 years while some cultivated bamboos like *Bambusa tulda, B. balooca* and *B. teres* rarely flower even in 100 years (Barooah, 2009). Therefore, molecular taxonomy is the only reliable option to ascertain the taxonomic status of bamboo. The commonly used methods are study of seed protein profile by SDS- PAGE which is recognized by International Seed Testing Association (ISTA) for cultivar identification and to characterize at species level (ISTA, 1996). But due to irregular and long flowering cycle bamboo seed is rarely available and hence study of seed protein profile is not practicable. In this background DNA marker based molecular approach is the only feasible option to resolve taxonomical status of available germplasm. Among the molecular markers, the most extensively used and convenient marker system is random amplified polymorphic DNA (RAPD). Finger printing genome using arbitrary primers was first demonstrated by Welsh and McClelland, (1990). Since then a large number of workers have successfully used this method to work out interspecific and intraspecific genetic variation among a large number of crop and other plant species.

North East India is recognized as one of the foremost center for bamboo biodiversity. At global level India harbor second highest diversity with 136 species belonging to 30 genera; next only to China. However, as per revised estimate (Barooah, 2009) global diversity comprise of 1250 species with 75 genera, while the corresponding figure for India are 129 species and 18 genera. More than 70% of India's biodiversity is found in North East India with 129 species belonging to 16 genera.

Despite this uncertainty prevail regarding taxonomic status of many taxa since they are arbitrarily assigned status based a morphological features which are highly influenced by local climatic condition and environmental changes. There is practically no information regarding taxonomic evaluation based on molecular approach. Against this background the present study was undertaken to study taxonomic status of 11 indigenous bamboo taxa from Assam and N.E India.

MATERIALS AND METHODS

Eleven species of bamboo were taken for the present study. These are *Bambusa arundinacea* (Kotah/kotoha), *B. tulda* (Jati banh), *B.balcooa* (Bhaluka), *B. teres* (Bhaluki Mokal), *B. vulgaris* (Bor jati), *B. pseudopallida* (Nol banh), *B. pallilda* (Bijuli banh), *Melocanna baccifera* (Tarza banh), *Dendrocalamus hamiltonii* (Bhoium kako), *D. giganteus* (Kako), *D.hamiltonii* (Paharia kako). Identification of bamboo species were done in collaboration with bamboo taxonomist Dr. C.K. Baruah of Assam Science Technology and Environment Council, Assam. Since two bamboos occurring in hill and plain were identified as *D. hamiltonii*, despite appreciable morphological difference they were designated as *D. hamiltonii* (H) for those occurring in hill and *D. hamiltonii* (P) for those occurring in plain

area. Total genomic DNA was isolated from fresh and young leaves using the CTAB (cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987) with few modifications. 200 mg of leaves were grounded in a pre chilled morter using liquid nitrogen followed by extraction with 1ml of CTAB extraction buffer (75 mM Tris–HCl; 100 mM EDTA, pH 8.0; 1.4 M NaCl, 2.5% w/v CTAB, 0.2% mercaptoethanol).

DNA was precipitated with ice cold isopropanol followed by washing with 70% ethanol. The pellets were dried and dissolved in 50 µl TE buffer and stored in -40°C till further use. DNA quality and quantity were checked in 0.8% agarose gel. A clear, dark orange thick band without smear along the lane was considered as good quality DNA and used for subsequent work. For further checking quality and quantity of the genomic DNA 5 µl DNA samples were taken and absorbance were recorded in a biophotometer (Eppendorf AG 22331) at 260 and 280 nm. The ratio for 260/ 280 nm were worked out and a ratio of 1.7 to 1.9 was considered as high purity DNA. DNA concentration was calculated with the following equation.

$$DNA\ (\mu g/\mu l) = Abs\ \lambda 260 \times 50 \times 100 \times (dilution\ factor)$$

Master mixture (25µl) was prepared with the following composition -17µl of sterile distilled water (Nuclease free water), 3µl of reaction buffer, 2.0µl dNTPs, 1µl of template DNA, 1µl of primer and 1µl Taq DNA polymerase. DNA amplification was carried out in a Thermocycler (Eppendorf, Mastercycler gradient) and the thermal cycle conditions for PCR reactions were an initial denaturation cycle of 1 minute and 30seconds at 94°C. Amplification was carried out as per the following schedule – 1 minute at 94°C for denaturation, 1 minute at 36°C for primer annealing, 2 minutes at 72°C for chain extension. The number of thermal cycles was set 40. An additional cycle of 7 minutes at 72°C was used for final extension. Amplification products were resolved by electrophoresis in 1.5% agarose gel. 8µl of 100bp DNA size marker (Bangalore Genei) was co-electrophoresed for size determination of DNA bands. The gel was run at 60 volts for 3 hours. Subsequently the gel was stained with ethidium bromide and visualized in UV-transilluminator. The gel image was recorded in gel documentation system (Gel Logic 212 PRO).

Table 1: Primer used for DNA amplification with specification

Sl. No.	Primer used	Primer sequence (5'-3')
1	RPI-6	5'....ACACACCGTG....3´
2	RPI-7	5´....ACATCGCCCA....3´
3	RPI-8	5'....ACCACCCACC....3'
4	RPI-9	5'....ACCGCCTATG....3'
5	RPI-10	5'....ACGATGAGCG...3'

Only clear, unambiguous bands were considered for scoring. The Em values of the amplicons for each lane were measured. For size determination of the amplicons a standard curve was prepared based on DNA size marker specified. From the standard curve the size

of individual amplicon was deduced and expressed in base pair (bp). Data were scored as 1 for presence and 0 for absence for that amplicon for a particular DNA sample. The binary data matrices were entered into the NTSYS PC package. The similarity matrix was prepared using Jaccard's similarity co-efficient (Jaccard and Paul, 1901, Jaccard *et. al*, 1990), which was used to generate dendrogram by Unweighted Pair Group Method with Arithmetical Average (UPGMA) with average linkage, using the software NTSYS PC v-2.02j.

RESULTS AND DISCUSSION

A total 5 primers were used to amplify genomic DNA and all the primers amplified variable number of amplicons. Among the primers the most impressive DNA profile was observed for primer RPI-10 which generated a total 28 bands in the size range of 180 bp to 180^0 bp (Table 2). It is noteworthy that no two DNA samples had identical profile. *M. baccifera* exhibited highest number of 11 DNA bands comprising of high, medium and low molecular weight amplicon followed by *D. hamiltonii* (H) and *B. teres* both with 10 DNA bands. Lowest number of 7 bands were observed in *B. arundinacea*. The amplicons with molecular weight 840 bp and 560 bp were found in highest frequency in as many as 8 species. On the other hand as many as 10 amplicons were found to occur only once and this signify the wide diversity prevalent among the bamboo species.

Primer RPI-6 also revealed impressive DNA profile with clear and well defined amplicons without ambiguity. RPI-6 generated a total 28 amplicons comprising of high medium and low molecular weight amplicons in the size range of 120 bp to 1800 bp. However the amplicon profile was different from RPI-10. No amplicon was found common to all the 11 species. Only 3 amplicons with molecular weight 1360 bp, 530 bp and 330 bp were found in highest frequency in 6 species. On the other hand 4 amplicons occurred only once. 3 species viz – *Bambusa tulda*, *Dendrocalamus hamiltonii* (H) and *D. giganteus* exhibited highest number of amplicons 9 each out of total 28 amplicons among all the species.

The other primers viz - RPI -7, RPI -8 and RPI-9 amplified 23, 18 and 26 amplicons. Apart from qualitative difference quantitative differences were also observed. For instance most of the high molecular weight amplicons had very low concentration of DNA as evident from the fact that they occurred as faint bands. On the other hand most amplicons for *Bambusa pallida* had comparatively high amount of DNA as evident from bright and thick coloured band. Likewise for primer RPI-6 the band with molecular weight 1250 bp appear to have highest concentration of DNA as evident from the fact that it was the brightest and thickest.

SI matrix and dendrogram based on RPI 10 revealed highest SI value between *Bambusa arundinacea* and *Bambusa balcooa* with 60 % followed by *Bambusa arundinacea* and *Bambusa teres* with SI value of 54.5%. On the other hand lowest value of 0.0% was found between *Dendrocalamus giganteus* and *Bambusa pseudopallida*. Among no two species SI value of 100% was observed. Dendrogram generated two major clusters with *Bambusa tulda*, *Dendrocalamus hamiltonii* (P) and *Dendrocalamus giganteus* forming one cluster while the others form the second and major cluster. Among the species *Dendrocalamus giganteus* is phylogenetically most distinctly related to the rest. Similarly *Melocanna baccifera* also

appears to be distantly related to the rest. Overall, dendrogram revealed wide genetic diversity as evident from wide Jaccard distance.

The advantage of DNA based marker is that unlike biochemical marker it is not affected by environment, external and internal factors except genetic factors. Moreover any tissue at any stage of growth and season can be used as source of material for DNA. The present study clearly shows that there exist wide genetic diversity among the bamboo species. DNA profile shows that unlike most food crops no two taxa among the 11 species have identical profile. SI matrix shows that out of possible 55 pair wise comparisons only 5.4 % pair show similarity little above 50% while for all others these are well below 50%. Dendrogram analysis shows two major clusters but within clusters all the taxa exhibit significant phylogenetic distances. Based on morphological features, conventional taxonomy consider as closely related and molecular analysis also support this view with SI value of 60%. In Assam as well as most part of NE India *B. tulda* and *B. balcooa* are most widely cultivated species but with distinct morphological difference. In the present study dendrogram analysis show that both belong to different cluster with wide genetic distance which lend support to the conventional taxonomic view. On the other hand taxonomic status of *D. hamiltonii* of hills and plain areas are a taxonomic puzzle. Conventional taxonomist consider both as the same species despite some morphological difference (Barooah, 2009). For this reason in the present study they were demarcated as *D. hamiltonii* (Plains), *D. hamiltonii* (Hill). The present study show that contrary to conventional taxonomic view both belong to separate clusters with wide genetic distance as evident from the fact that SI value between both is only 26.8%. Hence molecular analysis indicate that *D. hamiltonii* (P) and *D. hamiltonii* (H) are two separate species. On the other hand *D. giganteus* appear to be unrelated to all other taxa with SI value from 0.0% to 30.77%. These apart as many as 10 amplicons of diverse size were observed in only a single taxa. Generally an amplicon with single appearance is considered as unique band for the taxa concerned. From this view point it is evident that all the 11 taxa in the present are genetically distinct and also unique.

Table 2: Frequency distribution for amplicons generated with RPI 10 for the 11 taxa of bamboo.

Band [Em (cm)]	Molecular Wt. (bp)	*Bambusa arundinacea*	*B.tulda*	*B.balcooa*	*B. teres*	*B. vulgaris*	*B. pseudopallida*	*B. pallida*	*Melocanna baccifera*	*D. hamiltonii (H)*	*D. giganteus*	*D. hamiltonii (P)*	*Frequency for an amplicon*
1.9	1800	0	0	0	0	0	0	0	0	0	1	0	1
2	1650	0	0	0	0	0	0	0	0	1	0	0	1
2.1	1500	0	0	0	0	0	0	0	1	0	0	0	1

2.3	1360	0	0	0	0	0	0	0	0	1	1	0	2
2.35	1260	0	0	0	0	0	1	0	1	0	0	1	3
2.5	1220	1	1	1	1	0	0	0	1	1	1	0	7
2.6	1050	0	0	0	1	0	1	1	0	0	0	1	4
2.85	1000	0	1	0	1	1	1	0	0	1	0	1	6
2.9	920	0	1	0	0	0	0	1	1	0	1	0	4
3	870	0	0	0	0	1	0	0	0	0	0	0	1
3.05	840	1	0	1	1	1	1	1	1	0	0	1	8
3.2	820	0	1	0	0	1	0	1	0	1	1	1	6
3.3	800	1	0	1	1	0	0	0	0	0	0	0	3
3.4	720	0	0	1	0	0	1	1	1	0	0	1	5
3.5	680	0	1	0	0	0	0	0	0	1	0	0	2
3.6	650	1	0	1	1	1	0	1	1	0	0	1	7
3.7	615	0	0	0	0	0	0	0	0	0	1	0	1
3.8	600	0	0	0	1	0	0	0	0	0	0	0	1
3.9	560	1	1	1	0	1	1	1	0	1	0	1	8
4.1	490	0	0	0	0	0	0	0	0	0	0	1	1
4.2	470	1	1	1	1	1	1	0	1	0	0	0	7
4.4	440	0	1	1	0	0	0	0	0	0	0	0	2
4.5	420	1	0	0	1	1	0	0	1	1	1	1	7
4.6	400	0	0	0	0	0	0	1	0	0	0	0	1
5.1	260	0	0	0	0	0	0	0	0	0	1	0	1
5.4	220	0	0	0	0	0	0	0	1	0	1	0	2
5.65	180	0	0	0	0	0	0	0	1	0	0	0	1
For a taxa		7	9	9	10	9	8	9	11	8	9	10	

Table 3: SI matrix for the eleven taxa based on DNA profile amplified with RPI 10

	B. arundinacea	*B.tulda*	*B.balcooa*	*B. teres*	*B. vulgaris*	*B. pseudopallida*	*B. pallida*	*M. baccifera*	*D. hamiltonii* (P)	*D. giganteus*	*D. hamiltonii* (H)
B. arundinacea	100										
B.tulda	23.08	100									
B.balcooa	60.00	38.46	100								
B. teres	54.55	26.67	46.15	100							

B. vulgaris	45.45	38.46	38.46	46.15	100						
B. pseudopallida	25.00	30.77	41.67	38.46	41.67	100					
B. pallida	23.08	28.57	38.46	26.67	38.46	41.67	100				
M. baccifera	38.46	17.65	33.33	31.25	25.00	26.67	25.00	100			
D. hamiltonii (P)	25.00	41.67	13.33	20.00	30.77	14.29	13.33	11.76	100		
D. giganteus	14.29	20.00	5.88	11.76	12.50	0.00	12.50	25.00	30.77	100	
D. hamiltonii (H)	30.77	18.75	26.67	33.33	46.15	50.00	46.15	31.25	28.57	11.76	100

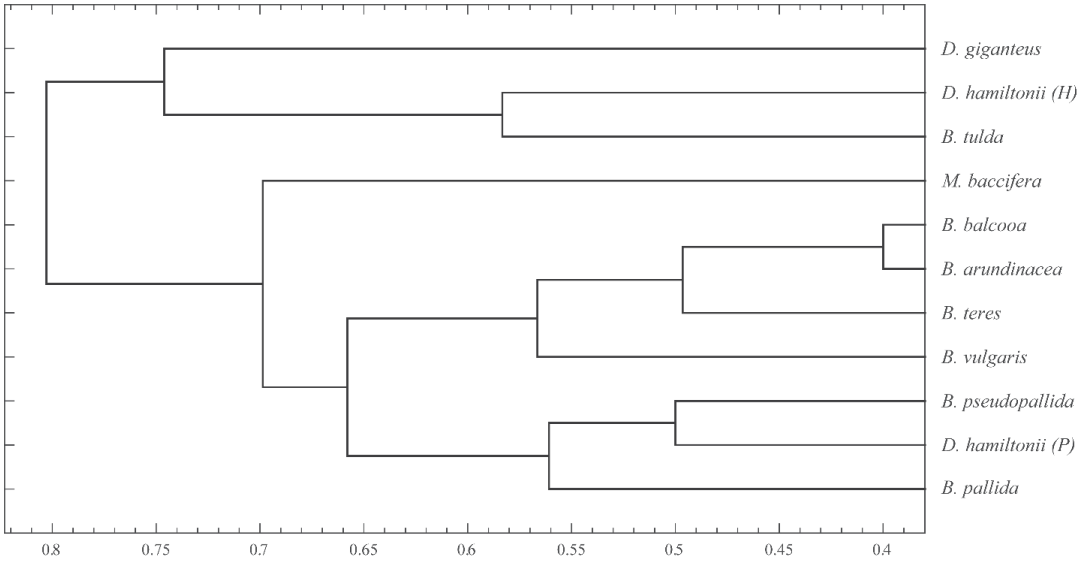

Fig.1. Dendrogram for the bamboo taxa based on DNA profile amplified with random primer RPI 10 using Jaccard distance

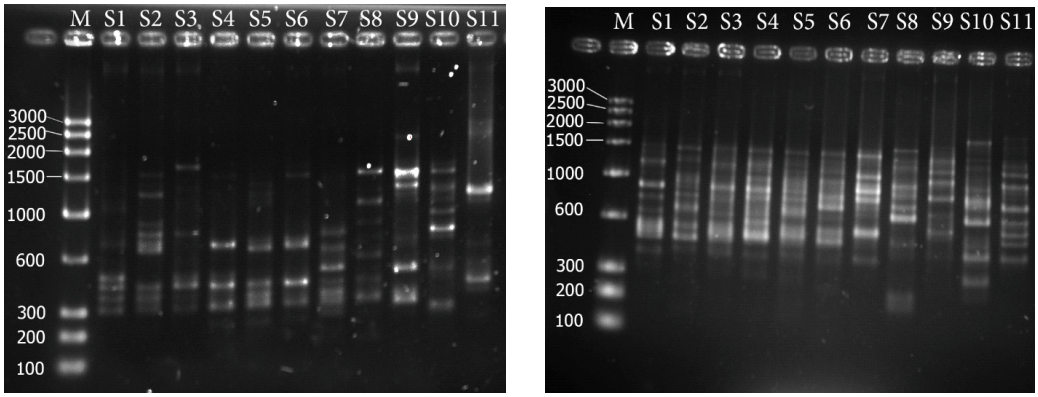

Fig. 2. Gel image for DNA profile amplified with RPI-6 (right) and RPI-10 (left)

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