BIOLOGIA PLANTARUM 51 (4): 667-672, 2007

# Phylogenetic relationships among the bamboo species as revealed by morphological characters and polymorphism analyses

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## Abstract

Phylogenetic relationships among 15 bamboo species were evaluated using 32 key morphological descriptors and 120 polymorphic loci of the genomic DNA generated using randomly amplified polymorphic DNA (RAPD) technique. The dendrogram generated for these two parameters based on similarity matrix computed from allelic polymorphism data using unweighted pair group method of analysis. Phylogenetic relationships as revealed from the dendrogram and principal component analysis was in concurrence with the reliable, widely referred system of bamboo classification, while, the cluster pattern generated from the similarity matrix derived from key morphological character analysis was discriminatory. The findings suggest that the molecular evidences need to be supplemented by morphological data to validate the phylogenetic relationships among taxa.

*Additional key words*: cluster analyses, culm, culm-sheath, RAPD.

## Introduction

India, being the second largest bamboo reserve in the world after China, still dependant on vegetative characters (culm and culm-sheath) for the identification mainly due to the unusually long sexual cycle and unavailability of any other diagnostic tool. While, vegetative characters are often influenced by the environmental factors (Wu 1962), hence, considered as less reliable for systematic studies (Ohrnberger 2002). Therefore, molecular technique based approaches were undertaken to establish phylogenetic relationships in temperate bamboos targeting either nuclear genome (Friar and Kochert 1994, Kobayashi 1997 and others) or nuclear r-RNA gene sequences (Hodkinson *et al*. 2000, Guo *et al*. 2001).

Since its discovery (Williams *et al*. 1990), random amplified polymorphic DNA (RAPD) technique has been successfully employed in the evaluation of genetic relationships in bamboo and other plant species (Gunter *et al*. 1996, Gielis *et al*. 1997, Nair *et al*. 1999, Nayak *et al*. 2003, Rout 2006). Padmesh *et al*. (2006) proved the potential use of RAPD to estimate intra-specific variation in *Mucuna pruriens* that could not be detected by sequence based ITS approach. Gielis *et al*. (1997) demonstrated that the RAPD approach could generate reliable markers that are useful for establishing species relationships in *Phyllostachys* and bamboos in general. For the identification of potential bamboo germplasm, Nayak *et al*. (2003) had used RAPD markers to study the genetic variations among 12 species of tropical bamboo. In this study we have employed two independent parameters, namely, 32 key morphological descriptors and 120 polymorphic loci in the genomic DNA to evaluate the phylogenetic relationships among 15 tropical bamboo species.

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*Received* 16 September 2005, *accepted* 22 July 2006.

*Abbreviations*: OTU - operational taxonomic unit; PCA - principal component analysis; PIC - polymorphism information content; RAPD - random amplified polymorphic DNA; UPGMA - unweighted pair group method of arithmetic averages.

*Acknowledgement*: The authors acknowledge the financial support of the Council of Scientific and Industrial Research [Grant No. 38 (1062)/03/EMR-II] and a fellowship to SB, New Delhi, India. The authors are thankful to the Director, Botanical Survey of India for various help including the supply of plant materials.

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## Materials and methods

**Plants:** Leaf samples from healthy donor plants of 15 bamboo species including *Bambusa affinis* Munro., *B.* arundinacea Retz., *B. atra* Lindl., *B. auriculata* Kurz., *B. balcooa* Roxb., *B. multiplex* 'Riviereorum' R. Maire, *B. oliveriana* Gamble, *B. striata* Lodd. ex Wendl., *B. tulda* Roxb., *B. vulgaris* Schrad. ex Wendl., *B. wamin*, Camus., *Dendrocalamus giganteus* Munro., *D. strictus* (Roxb.) Nees., *Gigantochloa atroviolacea* Widjaja and *Pseudobambusa kurzii* (Munro) Ohrnberger, were collected from the germplasm stock maintained at the Botanical Survey of India, Howrah, West Bengal, India. Five independent stands of each species were sampled at random for morphological and molecular analyses.

**Scoring of morphological descriptors:** Each species was considered as a separate independent operational taxonomic unit (OTU). Thirty-two key morphological descriptors (15 culm and 17 culm-sheath) were assessed from each of the 15 OTUs (5 replications per OTU) studied in the field (Tables 1 and 2). Mean values obtained from five independent replications were used as OTU representative data for each of the quantitative morphological descriptors. The scored qualitative and quantitative interval data were standardized to construct the dendrogram using unweighted pair-group method of arithmetic averages (UPGMA, Sneath and Sokal 1973) with *NTSYS-pc ver. 2.2* (Rohlf 2000).

**Isolation of PCR-compatible genomic DNA:** Genomic DNA was isolated from 0.2 g of sterilized leaf tissues using a modified protocol of Dellaporta *et al.* (Basak *et al*. 2004). Total RNA was removed by RNase treatment following the standard method. The concentrations of DNA samples were determined by comparing band intensity with known concentrations of lambda DNA by electrophoresis on 0.8 % agarose gel, stained with ethidium bromide and visualized under UV fluorescence.

**Detection of allelic polymorphisms:** To detect allelic polymorphisms within 15 bamboo species, 30 random, decamer primers were initially screened. The RAPD reactions were carried out in a 0.05 $cm^3$ reaction mixture following the protocol of Das *et al*. (2005). The thermal cycler programme used for amplification was: 4 min at 95 °C followed by 35 cycles (45 s at 94 °C, 45 s at 35 °C, 1 min at 72 °C) and finally 10 min at 72 °C for elongation. The amplified products were resolved by electrophoresis on 1.5 % agarose gel and TAE buffer. Gels were visualized by ethidium bromide staining and recorded with a gel doc 1000 camera using the programme Molecular analyst ver. 1.5 (*Bio-Rad*, Hercules, USA). The size of amplified DNA fragments (bands) was calculated by using imaging densitometer (model *GS-700*, *Bio-Rad*).

**Data scoring and RAPD profile analysis:** Polymorphisms in amplified products were scored for presence (1) or absence (0) of bands. Each amplification reaction, performed with genomic DNA isolated from five independent strands of each species and the reproducibility of reaction was repeated thrice. Only distinct and reproducible bands were recorded. A total of 122 amplified fragments, generated by 8 selected primers (Table 3) amongst 15 bamboo species were employed in further statistical analysis. The *NTSYS-pc* software package (version 2.2) was used to calculate pairwise genetic distances between the bamboo species (OTUs) based on coefficient of similarity (Nei and Li 1979). The UPGMA method was employed to compute the dendrogram from similarity coefficient values of OTUs. The relative discriminatory value of a primer was estimated by its polymorphism information content (PIC) value, and was calculated by using the equation:

$$PIC_i = 1 - \sum_{j=1}^{n} P^2 ij$$

where $P_{ij}$ is the frequency of the jth allele for the ith primer and summed over n number of alleles (Ni *et al*. 2002).

## Results and discussion

In the absence of flower or fruit characters, the culm-sheath (Raizada and Chatterjee 1956) and culm characters are treated as two major taxonomic keys for the identification of bamboos (Tables 1, 2).

The whole dendrogram was split into two clusters, *B. arundinacea* was totally isolated (Fig. 1). The main cluster was divided into two sub-clusters. All the eleven members of the genus *Bambusa* were split in different sub-clusters, except *B. striata* and *B. wamin*. The two *Dendrocalamus* species were distantly placed. *D. strictus* was grouped with *B. striata, B. wamin* and *B. atra,* while, *D. giganteus* clustered with *G. atroviolacea* and grouped with *B. affinis* and *P. kurzii*. The branching pattern of the dendrogram is not fully in agreement with the classical taxonomic classification of bamboos as proposed by Gamble (1896), according to which all the bamboo plants fall under the tribe *Bambuseae* of the family *Poaceae*. The genera *Bambusa* and *Gigantochloa* were included under the sub-tribe *Eubambuseae*, while the genus *Dendrocalamus* was included within the sub-tribe *Dendrocalameae*.

Thirty random primers were initially screened to generate polymorphic bands, of which, 8 produced distinct, reproducible, RAPD profiles among the 15 bamboo

Table 1. A comparison of the 15 key culm descriptors used to evaluate phylogenetic relationships among 15 bamboo species (OTUs): mean height [m], diameter [mm], length of 5th internode [cm], ratio of cavity diameter to total culm diameter, internode bending (absent = 0, present = 1), colour (yellow with striation = 0, yellow-green = 1, gray-green =2, pale green = 3, bright green = 4, glossy green =5, dark green = 6), swollen node (absent = 0, present = 1), nodal ring (absent = 0, whitish = 1, grayish = 2), nodal sheath scar, hairs at nodal ring, piercing culm sheath, different culm leaf and branch leaf size, curved lower nodal branches, different culm leaf and branch leaf size (absent = 0, present = 1), modification of branches (none = 0, thorns = 1, spines = 2), striation on culm (absent = 0, present = 1). 1 - *Bambusa atra*, 2 - *B. auriculata*, 3 - *B. arundinacea*, 4 - *B. balcooa*, 5 - *B. multiplex*, 6 - *B. oliveriana*, 7 - *B. striata*, 8 - *B. tulda*, 9 - *B. vulgaris*, 10 - *B. wamin*, 11 - *B. affinis*, 12 - *Pseudobambusa kurzii*, 13 - *Gigantochloa atroviolacea*, 14 - *Dendrocalamus giganteus*, 15 - *D. strictus.*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Height | 7.0 | 18.3 | 15.8 | 20.0 | 3.0 | 14.0 | 7.0 | 18.3 | 19.8 | 7.0 | 6.5 | 9.14 | 21.0 | 30.5 | 14.0 |
| Diameter | 35.0 | 45.0 | 55.0 | 90.0 | 20.0 | 37.5 | 70.0 | 50.0 | 80.0 | 120.0 | 40.0 | 35.0 | 60.0 | 130.0 | 50.0 |
| Internode | 60.0 | 40.0 | 23.0 | 20.0 | 20.0 | 35.0 | 15.0 | 38.0 | 23.0 | 12.0 | 50.0 | 22.0 | 20.0 | 33.0 | 37.5 |
| Cavity | 0.42 | 0.22 | 0.36 | 0.33 | 0.12 | 0.53 | 0.42 | 0.40 | 0.50 | 0.33 | 0.37 | 0.50 | 0.54 | 0.69 | 0.60 |
| Bending | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Colour | 6 | 3 | 2 | 4 | 3 | 5 | 0 | 6 | 4 | 6 | 2 | 2 | 6 | 2 | 1 |
| Swollen node | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Nodal ring | 1 | 2 | 0 | 1 | 2 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 3 | 0 |
| Sheath scar | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| Hairs | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Piercing sheath | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Curved branches | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Different size | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 |
| Modification | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Striation | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

Table 2. A comparative account of the 17 key culm-sheath descriptors used to evaluate phylogenetic relationships among 15 bamboo species (OTUs): ratio of total length to breadth at base, ciliate margin, pubescent adaxial side, pubescent abaxial side, hair colour (none = 0, golden brown = 1, brown = 2, dark brown = 3, black = 4), number of hairs (absent = 0, scanty = 1, profuse = 2), ratio of total length vs. blade length, shape of blade (triangular = 0, acuminate =1, lanceolate = 2, ovate = 3), blade reflexed, hairy margin of blade, ligule margin (entire = 0, dentate = 1, serrate = 2), hairs on ligule, auricle, auricle continuous with blade, bristles on auricle, auricle fringed, variable culm sheath size at different culm height. * - absent = 0, present = 1. 1 - *Bambusa atra*, 2 - *B. auriculata*, 3 - *B. arundinacea*, 4 - *B. balcooa*, 5 - *B. multiplex*, 6 - *B. oliveriana*, 7 - *B. striata*, 8 - *B. tulda*, 9 - *B. vulgaris*, 10 - *B. wamin*, 11 - *B. affinis*, 12 - *Pseudobambusa kurzii*, 13 - *Gigantochloa atroviolacea*, 14 - *Dendrocalamus giganteus*, 15 - *D. strictus.*

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length/breadth | 3.33 | 1.5 | 2.92 | 1.59 | 2.87 | 2.69 | 1.78 | 1.04 | 1.65 | 0.87 | 1.55 | 3.37 | 1.31 | 1.30 | 1.39 |
| Margin* | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| Pub. adax.* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pub. abax.* | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Hair colour | 1 | 4 | 4 | 4 | 0 | 4 | 3 | 4 | 3 | 2 | 4 | 4 | 1 | 2 | 1 |
| Hair number | 1 | 2 | 1 | 1 | 0 | 1 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 2 |
| Total/blade | 2.00 | 2.45 | 3.88 | 4.15 | 2.87 | 2.33 | 2.50 | 3.67 | 2.75 | 2.08 | 4.43 | 1.80 | 3.00 | 3.36 | 3.85 |
| Shape | 3 | 3 | 1 | 1 | 2 | 1 | 3 | 3 | 1 | 3 | 3 | 2 | 2 | 2 | 1 |
| Blade reflexed* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| Hairy margin* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 |
| Ligule margin | 0 | 1 | 1 | 2 | 0 | 2 | 2 | 2 | 0 | 2 | 0 | 1 | 0 | 2 | 2 |
| Hairs on ligule* | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| Auricle* | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| Auricle cont.* | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Bristles* | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Auricle fringed* | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sheath size* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 |

species surveyed. All these 8 primers generated a total of 122 amplified bands, among which 120 were polymorphic (Table 3). A very high frequency of polymorphism (98.36 %) was obtained. Nei (1978) estimated that a minimum number of 50 different loci are required for estimating genetic distances between species. While,

in the present investigation, the phylogenetic relationships were estimated using more than adequate number of loci, *i.e.* 120. A representative of RAPD profiles of 15 bamboo species generated with OPA-04 primer are depicted in Fig. 2.

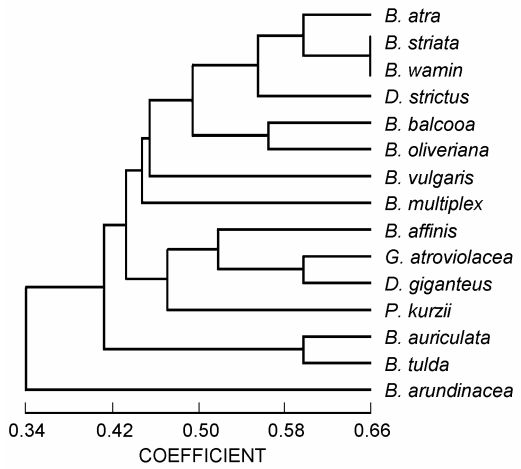


Fig. 1. Dendogram derived from UPGMA cluster analysis based on 32 key morphological characters of 15 bamboo species.

The size range of the amplified bands obtained was 209 to 2465 bp. The total number of bands per primer ranged from 9 (OPA-03) to 19 (OPA-04). PCR amplifications produced an average number of 15.25 scorable bands per primer, out of which, 15 bands were polymorphic (Table 3). In an analogous study, Nayak *et al*. (2003) have reported generation of 137 fragments from 12 bamboo species using 10 random primers and all these were reported to be polymorphic. Out of 8 polymorphism generating, reliable primers, PW-02 was found to be the most efficient primer with highest PIC (0.358) and the lowest value (0.292) was obtained with AP-01 primer. The PIC measures the marker system's ability to distinguish between genotypes (Weir 1990).

Similarity matrix obtained using Nei and Li's coefficient (data not shown) revealed that the range of similarity coefficient was 56.6 to 91.0 %. Although, similarity coefficients among the species of *Bambusa* are relatively high, yet, the *B. atra* formed a distinct clade, which perhaps indicative of their polyphyletic origin. Such polyphyletic origin of *Bambusa* was reported earlier (Loh *et al*. 2000). Dendrogram generated based on similarity

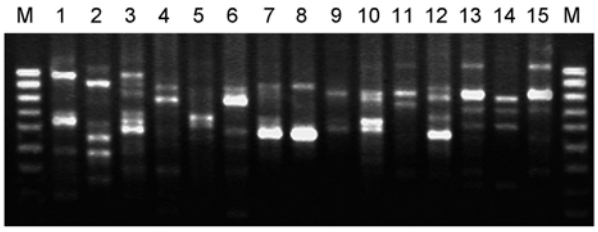


Fig. 2. A representative RAPD profile of 15 bamboo species amplified with OPA- 04 primer. Lane 1, *Bambusa atra*; Lane 2, *B. auriculata*; Lane 3, *B. arundinacea*; Lane 4, *B. balcooa;* Lane 5, *B. multiplex*; Lane 6, *B. oliveriana*; Lane 7, *B. striata*; Lane 8, *B. tulda*; Lane 9, *B. vulgaris*; Lane 10, *B. wamin*; Lane 11, *B. affinis*; Lane 12, *Pseudobambusa kurzii*; Lane 13, *Gigantochloa atroviolacea*; Lane 14, *Dendrocalamus giganteus*; Lane 15, *D. strictus*. M, 100 bp ladder marker.

indices computed from allelic polymorphism data obtained from RAPD profile analysis was split into two major clusters, excluding *B. atra* (Fig. 3). The remaining ten members of the genus *Bambusa* were grouped into one major cluster, while, the other four species under three genera grouped into another. The highest similarity coefficient (0.91) was obtained between *B. striata* and *B. vulgaris*. This observation complies with Bennet and Gaur's (1990) contention that *B. striata* is a somatic mutant of *B. vulgaris*. These two species were grouped with *B. wamin,* which was previously assigned as a variety of *B. vulgaris*. *B. balcooa* and *B. arundinacea* were found under the same minor cluster as reported earlier by Nayak *et al.* (2003). The dendrogram showed genetic closeness of *Gigantochloa* and *Dendrocalamus*. The similarity coefficient value between *G. atroviolacea* and *D. giganteus* was considerably high (0.861).

In the natural stands it is difficult to distinguish *B. tulda* from the *B. auriculata* and *D. giganteus* from the

Table 3. The numbers and size ranges of amplified and polymorphic bands generated with eight selected decamer primers and their respective PIC values.

| Primer | Nucleotide sequence (5´-3´) | Amplified bands | Polymorphic bands | Size range [bp] | PIC values |
|---|---|---|---|---|---|
| AP-01 | AGCCAAAGCC | 18 | 18 | 514 - 2465 | 0.292 |
| PW-02 | TCGTCGGCGT | 17 | 17 | 345 - 1464 | 0.358 |
| OPA-03 | AGTCAGCCAC | 09 | 08 | 300 - 1263 | 0.308 |
| OPA-04 | AATCGGGCTG | 19 | 19 | 390 - 1080 | 0.325 |
| OPA-05 | AGGGGTCTTG | 16 | 15 | 348 - 1774 | 0.334 |
| OPA-07 | GAAACGGGTG | 11 | 11 | 392 - 1812 | 0.326 |
| OPA-08 | GTGACGTAGG | 18 | 18 | 209 - 1706 | 0.313 |
| OPA-10 | GTGATCGCAG | 14 | 14 | 280 - 1347 | 0.350 |
| Total | | 122 | 120 | | |

*G. atroviolacea.* The phenotypic and genetic relatedness of these two species is also supported by both the dendrograms, reflecting their genetic closeness (Figs. 1, 3). The potentiality of RAPD technique to assess intra and inter specific genetic diversity of other bamboo species has already been demonstrated (Hsiao and Rieseberg 1994, Gielis *et al*. 1997, Nayak *et al*. 2003). This technique was employed by Gielis *et al*. (1997) to assess phylogenetic relationships among 73 genotypes of *Phyllostachys* (42 species and 31 infraspecific taxa). But the findings did not support the existence of two distinct groupings of *Phyllostachys* species complex, namely *Phyllostachys* and *Heteroclada* and the placement of *P. nigra* under *Phyllostachys* as advocated earlier by Friar and Kochert (1994) based on RFLP assay. To resolve this discrepancy, Ding (1998) employed RAPD based allelic polymorphism combined with the available morphological characters and concluded that *P. nigra* does not belong to the section *Heteroclada* as suggested by Gielis and coresearchers (1997). The above findings indicate that to validate the phylogenetic relationships among taxa, molecular evidences need to be supported by morpho-logical data.

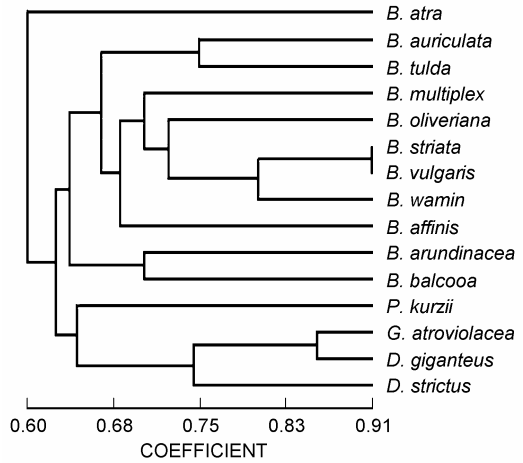


Fig. 3. Dendrogram derived from UPGMA cluster analysis based on 120 polymorphic alleles of 15 bamboo species.

Phylogenetic relationships amongst the 15 bamboo species revealed by the allelic polymorphism data is reasonably in concurrence with the taxonomic classification of Gamble (1896), while the cluster pattern obtained from the key morphological descriptors is not fully in agreement. This disagreement may be due to the fact that the classical system of classification is based on both, vegetative and reproductive characters; while only vegetative characters were analyzed in the present work due to the unavailability of reproductive organs.

The reliability of taxonomic groupings based only on the morphological characters has been often questioned due to the involvement of small number of genes for morphological traits that may not truly reflect the entire scenario of the genome (Brown-Guedira *et al*. 2000). However, in circumstances, where the full complement of morphological characters were not present or unobtainable, we have clearly demonstrated the potential use of allelic polymorphisms of the nuclear genome for the evaluation of phylogenetic relationships, which is also in congruence with the classical taxonomic classification of Gamble (1896).

It is apparent from this study that a solution to the long-standing problem of the systematics and identification of bamboo species is only possible through a multidisciplinary approach including DNA based polymorphisms.

## References

Basak, J., Das, M., Ghose, T.K., Pal, A.: An efficient and high-yielding method of PCR- compatible DNA isolation for biotechnological investigations from economically important plants. - In: D'Souza, L., Anuradha, M., Shashikiran, N., Hegde, S., Rajendra, K. (ed.): Biotechnology for a Better Future. Pp. 99-105. SAC Publications, Mangalore 2004.

Bennet, S.S.R., Gaur, R.C.: Thirty-seven Bamboos Growing in India. - Controller of Publications, Government of India, New Delhi 1990.

Brown-Guedira, G.L., Thompson, J.A., Nelson, R.L., Warburton, M.L.: Evaluation of genetic diversity of soybean introductions and North American ancestors using RAPD and SSR markers. - Crop Sci. **40**: 815-823, 2000.

Das, M., Bhattacharya, S., Pal, A,: Generation and characterization of SCARs by cloning and sequencing of RAPD products: A strategy for species specific marker development in bamboo. - Ann. Bot. **95**: 835-841, 2005.

Ding, Y.: Systematic studies on *Phyllostachys*. - Ph. D. Diss. Nanjing University, Nanjing 1998.

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

Gamble, J.S.: Bambuseae of British India. - Ann. Roy. Bot. Garden, Calcutta 1896.

Gielis, J., Everaert, I., De, L.M.: Analysis of genetic variability and relationships in *Phyllostachys* using random amplified polymorphic DNA. - In: Chapman, G. (ed.): The Bamboos. Pp.107-124. Academic Press, London 1997.

Gunter, L.E., Tuskan, G.A., Wullschleger, S.D.: Diversity among populations of switchgrass based on RAPD markers. - Crop. Sci. **36**: 1017 -1022, 1996.

Guo, Z.H., Chen, Y.Y., Li, D.Z., Yang, J.B.: Genetic variation and evolution of the alpine bamboos (*Poaceae, Bambusoideae*) using DNA sequence data. - J. Plant Res. **114**: 315-322, 2001.

Hodkinson, T.R., Renvoize, S.A., Chonghaile, G.N., Stapleton, C.M.A., Chase, M.W.: A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (*Bambusoideae, Poaceae*). - J. Plant Res. **113**: 259-269, 2000.

Hsiao, J.Y., Rieseberg, L.H.: Population genetic structure of *Yushania niitakayamensis* (*Bambusoideae, Poaceae*) in Taiwan. - Mol. Ecol. **3**: 201-208, 1994.

Kobayashi, M.: Phylogeny of world bamboos analysed by restriction fragment length polymorphisms of chloroplast DNA. - In: Chapman, G.P. (ed.): The Bamboos. Pp. 227-234. Linnean Society, London 1997.

Loh, J.P., Kiew, R., Set, O., Gan, L.H., Gan, Y.Y.: A study of genetic variation and relationships within the bamboo subtribe *Bambusinae* using amplified fragment length polymorphism. - Ann. Bot. **85**: 607-612, 2000.

Nair, N.V., Nair, S., Sreenivasan, T.V., Mohan, M.: Analysis of genetic diversity and phylogeny in *Saccharum* and related genera using RAPD markers. - Genet. Res. Crop Evol. **46**: 73-79, 1999.

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

Nei, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. - Genetics **89**: 583-590, 1978.

Nei, M., Li, W.: Mathematical model for studying genetic variation in terms of restriction endonucleases. - Proc. nat. Acad. Sci. USA **79**: 5269-5273, 1979.

Ni, J., Colowit, P.M., Mackill, D.J.: Evaluation of genetic diversity in rice subspecies using microsatellite markers. - Crop Sci. **42**: 601-607, 2002.

Ohrnberger, D., Goerrings, J.: The Bamboos of the World. - Elsevier Science, Amsterdam 2002.

Padmesh, P., Reji, J.V., Jinish Dhar, M., Seeni, S.: Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD. - Biol. Plant. **50**: 367-372, 2006.

Raizada, M.B., Chatterjee, R.N.: World distribution of bamboos with special reference to the Indian species and their more important uses. - Indian Forest. **82**: 215-218, 1956.

Rohlf, F.J: NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.2. Exeter Software, Setauket - New York 2000.

Rout, G.R.: Evaluation of genetic relationship in *Typhonium* species through random amplified polymorphic DNA markers. - Biol. Plant. **50**: 127-130, 2006.

Sneath, P.H.A., Sokal, R.R.: Numerical Taxonomy. - W.H. Freeman and Co., San Francisco 1973.

Weir, B.: Genetic Data Analysis: Methods for Discrete Population Genetic Data. - Sinauer Assoc., Sunderland 1990.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. - Nucl. Acids Res. **18**: 6531-6535, 1990.

Wu, M.C.Y.: Classification of *Bambuseae* based on leaf anatomy. - Bot. Bull. Acad. sin. **3**: 83-107, 1962.