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Analysis of Genetic Variation in Selected Bamboo Species using RAPD

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ABSTRACT

Taxonomic and systematic studies of bamboos are traditionally based on floral morphology, which can cause problems in identification due to the lack of or infrequent flowering. There are limited studies using molecular techniques to overcome taxonomic problems in woody bamboos. In this study RAPD was used to differentiate 26 bamboo species. Screening was done with 50 random primers and consistent results were obtained with about 10. *Dendrocalamus* species stood out among other bamboo species.

Keywords: cluster analysis, *Dendrocalamus*, random primers

INTRODUCTION

Bamboos are vital to many Asian economies, having important uses ranging from domestic items to rural housing and raw materials for industry. The overexploitation and genetic erosion of bamboo species have made it necessary to preserve germplasm (Ramanayake 2006), whose classification and identification requires greater attention.

The main problem in bamboo species identification is the lack of basic knowledge of the biology and genetics of bamboo. This is the direct result of the unusual life cycle of bamboo. Among bamboo species, the vegetative growth varies from 1 to as much as 120 years and some species have never been known to flower (Ramachandran *et al*. 2007). Taxonomic studies that rely on floral morphology are therefore limited. The bamboo inflorescence is an area of morphology with serious problems of interpretation. The inflorescence type, somelauctant and interauctant, that has been used in defining genera is not easy easy to interpret (Li 1997). Although Stapleton (1997) forwarded a modern interpretation, it has not been widely adopted as it radically alters the traditional terminology. According to Watson and Dallwitz (1992), description of bamboo species will not be possible until the descriptive terminology of inflorescence and spikelet morphology are standardized.

The vegetative character used for identification are likely to be subtle and variable in most cases, it is difficult to say how much they reflect the true evolutionary history of these plants (Wu 1962; Ohrnberger 2002). Therefore, molecular-based approaches were undertaken to establish phylogenetic relationships in temperate bamboos targeting either the nuclear genome (Friar and Kochert 1994; Kobayashi 1997) 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 several plant species, including bamboo (Gielis *et al*. 1997; Nayak *et al*. 2003; Rout 2006; Das *et al*. 2006; Ramanayake *et al*. 2007).

In this paper, we demonstrated the use of RAPD in bamboo identification as well as determining genetic diversity and relationships between selected 26 bamboo species.

MATERIALS AND METHODS

Plant material

One-month-old seedlings of eight Bambusa species (*Bambusa bambos*, *B. pallida*, *B. tulda*, *B. balcooa*, *B. vulgaris*, *B. nutans*, *B. mugalba*, *B. polymorpha*) eight *Dendrocalamus* spp. (*Dendrocalamus strictus*, *D. hamiltoni*, *D. giganteus*, *D. hookeri*, *D. sikkimensis*, *D. asper*, *D. brandisii*, *D. stocksii*), three *Ochalandra* spp. (*Ochalandra ebracteata*, *O. scriptoria*, *O. travancorica*), two *Thyrostachys* spp. (*Thyrostachys oliveri*, *T. siamensis*), *Melocanna baccifera*, *Teinostachyum dullooa*, *Schizostachyum dullooa*, *Oxytenanthera stocksii* and *Phyllostachys bambusoides* were obtained from various institutes such as GB Pant University of Agriculture and Technology, Indian Forest Genetics and Tree Breeding Institute, Coimbatore, Forest College and Research Institute, Metupalayam.

DNA isolation and estimation

Terminal leaves of these seedlings were used for DNA extraction according to the method of Krishna and Jawali (1997). Two independent extractions were performed on different days for each species. The quantity of DNA was estimated by agarose gel electrophoresis and towards the latter part of the study by a Hofer DNA Quant 200 Fluorometer.

Biochemicals

Taq DNA polymerase and dNTPs were obtained from Bangalore Genei Pvt. Ltd. (Bangalore, India). Random primer kits of OPM, OPR, OPH, OPO, OPS and OPB series were obtained from Operon Technologies (Almeda, CA, USA). Other chemicals used were purchased locally and were of analytical grade.

DNA amplification

The DNA amplification mixture (25 µl) contained 25 ng template DNA (2 µl), 2.5 µl buffer, 1.0 µl primer, 0.25 µl dNTPs, 0.2 µl *Taq* Pol and 19.05 µl sterile water. PCR components were prepared as a master mix for each primer to minimize pipetting errors. Amplification were performed in two models of thermal cyclers: Model Gene Amp PCR System 2400 (Perkin Elmer, USA) and PTC-100 TM (MJ Research Inc., USA). Thermal cycle: 35 cycles

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Research Note

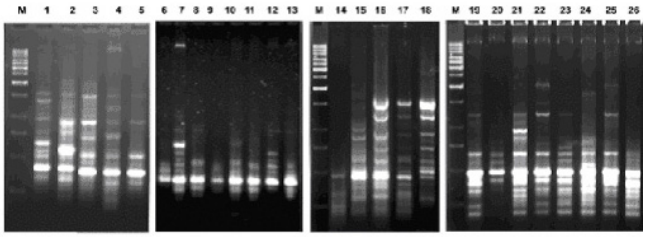

Fig. 1 Molecular profile of 26 bamboo species using random primer OPH-19. Lanes: 1. *Bambusa pallida*; 2. *B. balcooa*; 3. *B. nutans*; 4. *B. mugalba*; 5. *B. vulgaris*; 6. *Oxytenanthera stocksii*; 7. *Denderocalamus giganteus*; 8. *D. hookeri*; 9. *Schizostachyum dullooa*; 10. *Denderocalamus brandisii*; 11. *D. hamiltoni*; 12. *D. stocksii*; 13. *D. strictus*; 14. *Teinostachyum dullooa*; 15. *D. sikkimensis*; 16. *B. polymorpha*; 17. *B. bambos*; 18. *B. tulda*; 19. *Thyrsostachys siamensis*; 20. *D. asper*; 21. *Melocana bacciferra*; 22. *Thyrsostachys oliveri*; 23. *Ochalandra travancorica*; 24. *O. ebracteata*; 25. *Phyllostachys bambusoides*; 26. *Ochalandra scriptoria*.

with initial strand separation at 94°C for 3 min, followed by annealing at 37°C for 1 min and extension at 72°C for 1 min. After 35 cycles there was a final extension at 72°C for 10 min. Amplification products were electrophoresed in 1.6% agarose gel and stained with ethidium bromide. Each set of reactions was repeated in triplicate and only the reproducible bands were included in the analysis.

Statistical analysis

Evaluation of variation in the RAPD profile was performed by calculating the individual band frequency for each species. Polymorphism was scored for the presence (1) or absence (0) of bands. Cluster analysis was performed on the similarity matrix based on Jaccard's similarity index by the UPGMA method. All computations were performed with NTSYS-PC version 2.1 (Rohlf 1993).

RESULTS AND DISCUSSION

Fifty, 10-mer random primers of OPM, OPH, OPO, OPR, OPS and OPB series were tested for their ability to produce polymorphic bands. Primers resulting in monomorphic, too complex and irreproducible patterns were excluded from the studies. Ten primers were selected for further studies. The total number of scorable bands amplified using these 10 primers was approximately 58. Thus the average number of bands amplified per primer was six. The number of scorable bands generated per primer varied between 6 and 9 of the 58 fragments amplified, and 39 bands were polymorphic. The number of polymorphic bands generated per primer varied between two to eight. Of the 10 random primers, the percentage polymorphism was greatest in OPH 19 (88.88%) (**Fig. 1**), followed by OPM 16 (75.01%). Least polymorphism was found in OPO 10 (33%). Cluster analysis (**Fig. 2**) was performed based on the banding pattern obtained utilizing all 10 primers. Species like *D. stocksii* and *O. stocksii* were clustered together. Similarly, *D. strictus*, *D. hamiltoni* and *D. asper* had similar banding patterns and thus they appeared in a single cluster. In case of *Bambusa* species, *B. nutans*, *B. vulgaris* and *B. mugalba* showed similarity and are inferred as closely related species.

RAPD results support the position of *Thyrsostachys* within Bambusinae as a genus distant from *Bambusa*. This result is also confirmed by the AFLP studies (Loh 2000). The two different clusters of *Bambusa* spp. suggest that the genus *Bambusa* is polyphyletic and highlights the potential of RAPD to assess the variation and relationships within this genus. The two *Dendrocalamus* spp. examined were very different to *D. brandisii* falling within the *Bambusa* cluster. *D. giganteus* showed the least genetic similarity to any of the *Bambusa* species examined. The relationship between these species has been studied and split into several genera (Li 1997). Currently the genus *Dendrocalamus* is used in a broad sense (Li 1997; Wong 1995). At one extreme, some species are closely similar to species of *Bambusa* and there are also intermediate species (Li 1997).

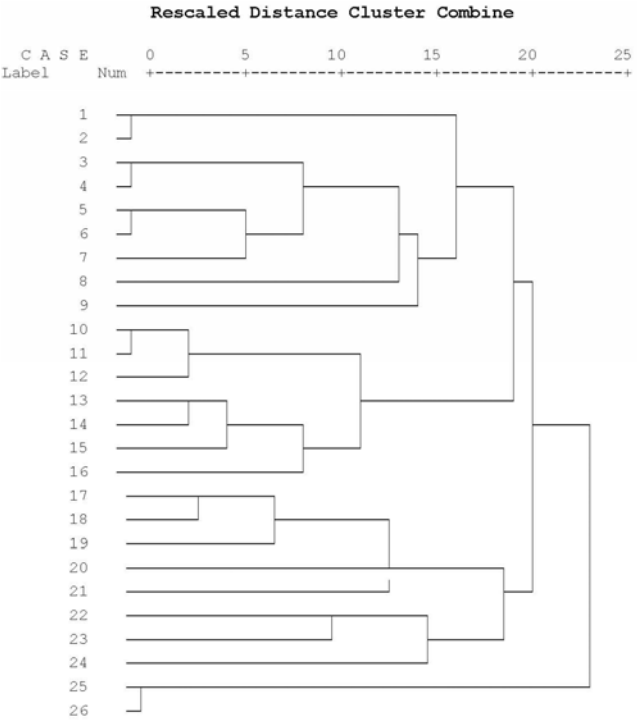

Fig. 2 Dendrogram of 26 bamboo species based on RAPD analysis. 1. *Oxytenanthera stocksii*; 2. *Denderocalamus stocksii*; 3. *D. strictus*; 4. *D. hamiltoni*; 5. *D. asper*; 6. *D. hookeri*; 7. *D. sikkimensis*; 8. *O. scriptoria*; 9. *O. travancoria*; 10. *O. ebracteata*; 11. *Thyrsostachys siamensis*; 12. *T. oliveri*; 13. *Phyllostachys bambusoides*; 14. *D. giganteus*; 15. *Melocana bacciferra*; 16. *Teinostachyum dullooa*; 17. *B. nutans*; 18. *B. vulgaris*; 19. *B. mugalba*; 20. *B. balcooa*; 21. *B. pallida*; 22. *B. tulda*; 23. *B. bambos*; 24. *B. polymorpha*; 25. *D. brandisi*; 26. *Schizostachyum dullooa*.).

Our study confirms the wide range of variation within *Dendrocalamus*, with *D. brandisii* clustering closely within the *Bambusa* cluster, while *D. giganteus* clustering very distantly from the rest of the *Bambusa*.

Wong (1995) studied two distinct groups within the genus *Dendrocalamus*, based on the combination of vegetative characters, inflorescence morphology and flowering behavior. Wong (1995) also cited the work of Chou and Huang (1985), whose study was on the chromatographic separation of phenolic compounds and electrophoretic isozymes patterns in *Dendrocalamus*. As Wong (1995) noted, *Dendrocalamus* requires further critical study.

In conclusion, RAPD was useful in studying the genetic similarity between bamboo species and could be used in the future to identify molecular markers specific for particular species.

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