International Journal of Genetics
ISSN: 0975-2862 & E-ISSN: 0975-9158, Volume 10, Issue 10, 2018, pp.-526-529.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000226

Research Article

PHYLOGENETIC ANALYSIS OF BAMBOOS THROUGH MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS

BHANDARI M.S.*[1], MEENA R.K.1, RAMA KANT1, KAUSHAL R.[2] AND TEWARI S.K.[3]
[1]Division of Genetics & Tree Improvement, Forest Research Institute, Dehradun, 248 195, India
[2]Division of Plant Science, Central Soil and Water Conservation and Research and Training Institute, Dehradun, 248 195, India
[3]Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, 263 145, Uttarakhand, India
*Corresponding Author: Email - maneesh31803@gmail.com

Received: July 31, 2018; Revised: September 26, 2018; Accepted: September 27, 2018; Published: October 30, 2018

Abstract- Phylogenetic relationships among 14 species of bamboo were established on the basis of morphological and biochemical parameters. Based on the pooled data analysis, Euclidean phenogram classified forteen species into five hierarchical clusters. Cluster IAaa had maximum number of species (7), cluster IAab had four species whereas cluster IAb comprises of *Bambusa tulda*, cluster IB had *Bambusa multiplex* and Cluster II had *Bambusa vulgaris*. The Euclidean distance coefficient was ranged from 0.449 to 2.242. The minimum genetic distance (0.449) was recorded between *Melocanna baccifera* and *Bambusa bambos*, while the maximum genetic distance (2.276) was found between *Bambusa vulgaris* and *Bambusa multiplex*. The phylogenetic relationships based on the dendrogram and principal component analysis was in accordance with the morphological bamboo classification system.

Key words- Cluster analysis, genetic diversity, biochemical parameters, Euclidean Phenogram

Citation: Bhandari M.S., *et al*., (2018) Phylogenetic Analysis of Bamboos through Morphological and Biochemical Parameters. International Journal of Genetics, ISSN: 0975- 2862 & E-ISSN: 0975-9158, Volume 10, Issue 10, pp.-526-529.

Introduction

India is having the second largest bamboo reserve after China. The phylogenetic analysis is still dependant on vegetative or morphological characters for the identification of bamboos mainly due to the unusually long sexual cycle and unavailability of any other diagnostic tool. Besides assessing genetic diversity, the purpose of germplasm characterization is to identify unique genotypes or genetically diverse genotypes for species improvement [1-4]. Since very less research occurs in the phylogenetic assessment, therefore, present study has been conducted on the morphological and biochemical parameters to revealed taxa-based clustering among bamboos.

Materials and Methods

Location of the Trial

The experimental material consisted of 14 species of bamboos *viz. Bambusa bambos, Bambusa balcooa, Bambusa multiplex, Bambusa nutans, Bambusa tulda, Bambusa vulgaris, Dendrocalamus giganteus, Dendrocalamus hamiltonii, Dendrocalamus longispathus, Dendrocalamus membraneceous, Dendrocalamus strictus, Gigantochloa albociliata, Melocanna baccifera,* and *Pseudoxytenanthera stocksii,* and were collected from the Bamboo Multilocation Trial, Pantnagar, Uttarakhand, India (Fig-1). The experiment was conducted in a completely Randomized Block Design (CRBD) with three replications. Each species was propagated in a single row with 5 culms. Culms of each species were maintained at 5 m distance between rows and columns. Three competitive culms were selected in all the three replications to study the various morphological and biochemical parameters.

Data Recording

Each bamboo species was considered as an independent operational taxonomic unit (OTU). Ten morphological characters and 8 biochemical parameters were assessed from each of the 14 OTUs (3 replications per OTU). Mean values obtained from 3 independent replications were used as OTU representative data for each parameter. Also, biochemical parameters were analysed in the laboratory by taking replicated samples from the trial for obtaining mean values of OTU. Data were recorded in total of 18 parameters *viz*., culm height, biomass, number of old shoots, number of new shoots, circumference of clump, diameter of old culm, diameter of new culm, inter-node length, leaf:stem ratio, dry matter percentage, crude protein content, crude fibre, neutral detergent fibre, acid detergent fibre, acid detergent lignin, dry matter digestibility, HCN content and silica content.

Data Scoring and UPGMA Analysis

The scoring was done for qualitative and quantitative parameters in which data were standardized to construct the dendrogram using unweighted pair-group method of arithmetic averages (UPGMA) [5], with NTSYS-pc ver. 2.2 [6]. The NTSYS-pc software package was used to calculate pairwise genetic distances between the bamboo species (OTUs) based on coefficient of similarity [7].

Results and Discussion

Bamboo taxonomy, which is based on the floral morphology and growth habit, is prone to mis-identification due to erratic flowering [8]. Therefore, the culm sheath [9] and culm characters became the major keys for taxon identification in bamboos [10]. In the present study, the Euclidean distance coefficients based on morphological and biochemical data were estimated and presented in Table-1. The minimum distance (0.449) was found between *M. baccifera* and *B. bambos*, while maximum genetic distance (2.276) analyzed between *B. vulgaris and B. multiplex*. The morphological variability phenogram based on Euclidean distance and UPGMA clustering was constructed (Fig-2). Phenogram separated 14 species into two major clusters *viz*. I and II at 2.12 distance coefficient. All bamboo species were placed in cluster-I, except *Bambusa vulgaris* which is the most divergent

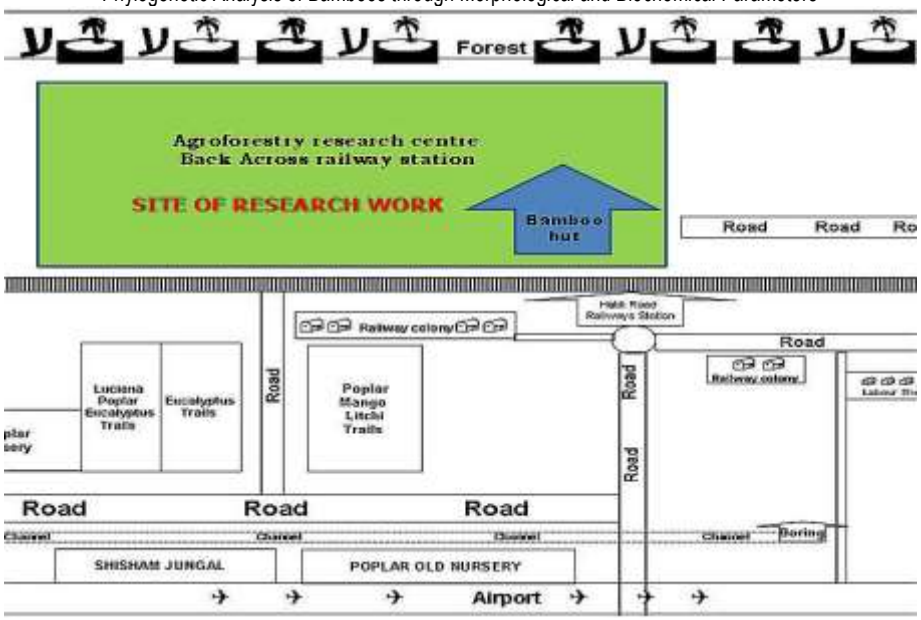

Fig-1 Experimental Site at Pantnagar, U.S. Nagar, Uttarakhand, India

Table-1 Euclidean coefficient of bamboos based on UPGMA phenogram

	Bbs	Bb	Bm	Bn	Bt	Bv	Dg	Dh	Dl	Dm	Ds	Ga	Mb	Ps
Bbs	-													
Bb	0.753	-												
Bm	1.026	1.215	-											
Bn	1.118	1.026	1.402	-										
Bt	1.035	1.251	1.538	1.231	-									
Bv	2.044	2.172	2.276	2.242	1.960	-								
Dg	0.915	0.792	1.352	0.658	0.934	2.214	-							
Dh	1.151	0.767	1.407	1.295	1.362	2.145	2.214	-						
Dl	0.868	0.778	1.311	0.857	1.522	2.117	2.145	1.076	-					
Dm	0.799	0.870	1.012	1.428	1.016	2.052	2.117	0.857	1.285	-				
Ds	1.117	0.966	1.144	1.247	1.295	2.142	2.052	0.601	1.171	0.681	-			
Ga	0.706	0.906	1.339	1.049	1.144	1.843	2.142	0.902	0.713	1.008	0.979	-		
Mb	0.449	0.699	1.063	0.938	0.983	2.038	1.843	0.895	0.798	0.727	0.823	0.536	-	
Ps	1.080	1.262	1.179	1.457	1.462	2.248	1.423	1.016	1.290	0.967	0.780	0.905	0.843	-

Bbs = Bambusa bambos, Bb = Bambusa balcooa, Bm = Bambusa multiplex, Bn = Bambusa nutans, Bt = Bambusa tulda Bv = Bambusa vulgaris, Dg = Dendrocalamus giganteus, Dh = Dendrocalamus hamiltonii, Dl = Dendrocalamus longispathus, Dm = Dendrocalamus membraneceous, Ds = Dendrocalamus strictus, Ga = Gigantochloa albociliata, Mb = Melocanna baccifera and Ps = Pseudoxytenanthera stocksii

amongst all the 14 species as it is separated from others at first fall, into cluster-II. Cluster-I was further bifurcated at 1.45 coefficient value into two subclusters (IA and IB) and cluster-IB comprised of only *B. multiplex*. Subcluster-IA subdivided into two subgroups, IAa and IAb at 1.10 distance coefficient, where subgroup IAb consisted of *B. tulda*. At 1.08 Euclidean coefficient value, subgroup IAa bifurcated into two sub-subclusters *viz*., IAaa and IAab. Sub-subclusters IAaa and IAab comprised of 7 and 4 species, respectively. Sub-subcluster IAaa was the most diverse among all the clusters, as genera and species *viz*., *B. bambos, M. baccifera, G. albociliata, B. balcooa, D. longispathus, B. nutans* and *D. giganteus* under this cluster had diverse origin. Sub-subclusters IAaa and IAab showed that geographical diversity could be used as a criterion of genetic diversity, since species in these two clusters belong to two common genera *viz*., *Bambusa* and *Dendrocalamus*. Thus, a peruse of the clustering pattern indicated that although geographical diversity is not always a symbol of genetic diversity, the effect of former can't be underestimated [11]. The principal component analysis was derived by a biplot, which incorporated both the phenotypic characters measured during field work and the biochemical parameters for fodder quality estimation from the laboratory experiments (Fig-3). Three-dimensional scatter plots of 14 species of bamboo were presented in Fig-4. Using the GGE biplot software, 5 main groups was observed [12].

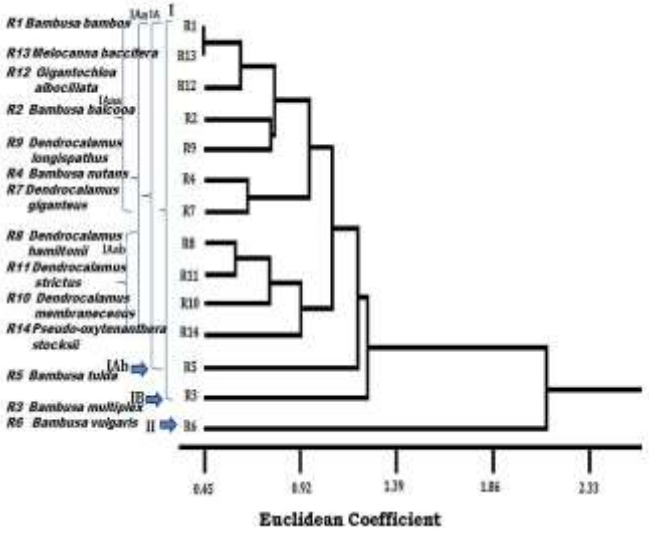

Fig-2 Euclidean coefficient and UPGMA clustering of bamboos

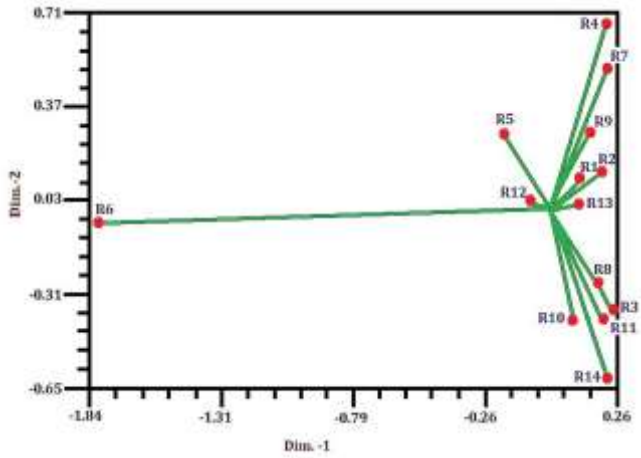

Fig-3 Principle component analysis of morphological data generated scatter plot (two-dimension)

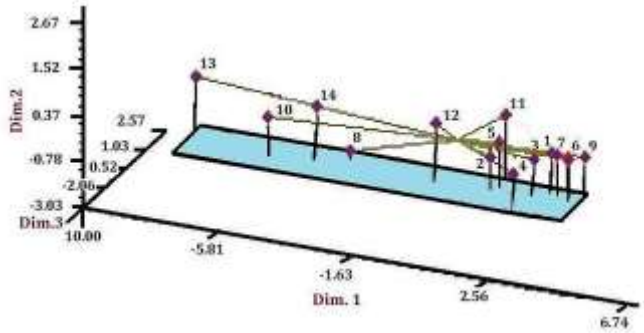

Fig-4 Principle component analysis of morphological data generated scatter plot (three-dimension)

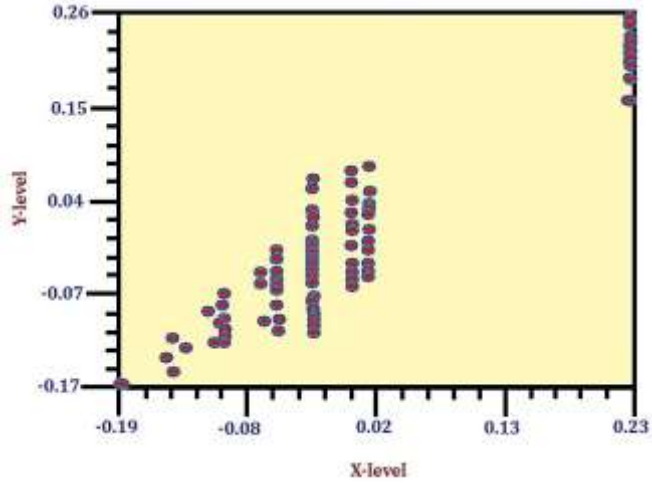

Fig-5 Relationship between similarity matrix and cluster analysis

Thus, total species can be classified at 1.06 euclidean coefficient values into five hierarchical clusters *viz*. IAaa, IAab, IAb, IB and II. Matrix comparison was performed using Mx COMP programme of NTSYS-pc to calculate the cophenetic correlation between the distance matrix and the cophenetic value matrix. The cophenetic value matrix generated from SAHN's cluster analysis was used to test two-way Mantel test. The estimated cophenetic correlation (r) was 0.933, indicating good fit of the cluster analysis and showed significant correlation between distance matrix and cluster analysis. The relationship between two matrices was depicted in Fig-5. Therefore, the dendrogram and the principal component analysis showed significant differences between the species of bamboo (Fig-2). Thus, suggested virtually lot of genetic diversity/variation among the genetic resources of bamboo species in the country. Recently, in Brazil, six species of bamboo *viz*., *Bambusa vulgaris, Bambusa vulgaris* var. vittata, *Drepanostachyum falcatum, Dendrocalamus latiforus, Phyllostachys aurea var. albovariegada* and *Phyllostachys edulis* were characterized using fifteen qualitative and nine quantitative descriptors. The methods of grouping of Tocher's and UPGMA was used to estimate the genetic divergence between the clones through Euclidean distance and the principal component in two-dimensional plane. The six studied species were easily differentiated by the qualitative and quantitative descriptors [13]. The five *Dendrocalamus* species were distantly placed. *Dendrocalamus longispathus* and *D. giganteus* were grouped *with B. bambos, B. balcooa, B. nutans, M. baccifera* and *G. albociliata* in cluster IAaa. While, D. *hamiltonii, D. membraneceous, D. strictus* and *P. stocksii* formed the separate cluster IAab. The clustering pattern of the dendrogram is not completely in-agreement with the classical taxonomic classification of bamboos, according to which all the bamboo plants fall under the tribe Bambuseae of the family Poaceae [14]. The genera *Bambusa* and Gigantochloa were included in the sub-tribe Eubambuseae, while the genus *Dendrocalamus* was included within the sub-tribe Dendrocalameae. 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 and biochemical parameters were analysed 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 [15]. However, with the full complement of morphological characters it is easy to establish phylogenetic relationships, which is also in congruence with the classical taxonomic classification. Classification and characterization of bamboos may vary based on the morphological and biochemical parameters only. But now a days, DNA based molecular markers techniques were used in support of classical taxonomic keys to demarcate the bamboo species distribution pattern and evolution [16]. Recently, much emphasis was given on Phyllostachys clade and related clades and 36 temperate bamboos and 3 outgroups (tribe Arundinarieae, Poaceae) were studied using restriction-site associated DNA sequencing (RAD-seq). To study the phylogenetic relationships with unparalleled resolution, especially for phylogenetically challenging group, the largest data matrix was used [17]. Similarly, maximum parsimony and Bayesian inference methods was used to study 53 species representing 17 paleotropical woody bamboos. The taxa were clustered into two clades, *i.e*., the Bambusinae + Dinochloa clade and the Melocanninae clade [18, 19]. Interestingly, the phylogeneticic analysis through molecular data in bamboos always not provide the best results. Estimation of molecular phylogeny of Chusquea (Poaceae: Bambusoideae: Bambuseae) presented weak molecular support for relationships within Euchusquea. Further, the absense of synapomorphic morphological characters for defining clades emphasize the use of morphology-based taxonomy for estimating diversity in the *Euchusquea clade*. The taxonomic description of different closely related bamboo species would rather be supported by the morphological, anatomical, ecological and sequence-based data [20]. One such study using morphological and plastidrpl16 intron sequence data of 43 species of Bambuseae, three of Olyreae (herbaceous bamboos), and two outgroup taxa suggested homoplasy between the morphological characters of Chusqueinae and Hickelinae, suggesting robust resolution of relationships among the major lineages of woody bamboos is still wanting [21]. Similarly, analysis of the North American *Arundinaria* species *complex, A. gigantean, A. tecta, and A. appalachiana* through AFLPs and chloroplast DNA sequences detected diversity within and among all three species plus individuals with intermediate or unusual morphological characteristics (putative hybrids) [22]. Molecular phylogeny of Japanese dwarf bamboos using RAPD and morphological data revealed association between the morphology and RAPD tree topology [23].

Conclusion

It could be concluded that for characterizing germplasm of bamboos which is a taxonomically difficult group for identification requires holistic component of research base. It includes morphological traits, biochemical parameters, reproductive components and DNA based barcoding techniques along with the associated species composition in the native range, since the genetic base is

much broader in bamboos as it comprises of large number of species. Potentially, bamboos are yet to be harnessed up to the maximum level as there is non-identification of proper source species of bamboo in particular climatic conditions. Moreover, improving agronomic practices might be needed; developing quality planting material will be a requirement for yield (culm as well as biomass) improvement [24].

Application of Research: Phylogenetic analysis would be helpful in knowing evolutionary significance of bamboos. Identification of unique genotypes or genetically diverse genotypes in bamboos.

Research Category: Phylogenetic characterization of Bamboos

Abbreviations:
OTU: Operational Taxonomic Unit
RAPD: Random Amplified Polymorphic DNA
UPGMA: Unweighted Pair-Group Method with Arithmetic averages

Acknowledgement / Funding: Authors are thankful to National Bamboo Mission, New Delhi, India, for providing financial support. Author also thankful to G.B. Pant University of Agriculture and Technology, Pantnagar, 263 145, Uttarakhand, India

Research Guide or Chairperson of Research: Dr Salil K. Tewari
University: G.B. Pant University of Agriculture and Technology, Pantnagar, 263 145, Uttarakhand, India
Research project name or number: Ph.D. Thesis

Author Contributions: All authors equally contributed.

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical Approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Ganesh Ram S., Parthiban, K.T., Senthil Kumar R., Thiruvengadam V. and Paramathma M. (2008) *Genetic Resources Crop Evol*., 55: 803–809.
[2] Kapteyn J. and Simon J.E. (2002) *In: Janick J., Whipkey A. (Eds.) Trends in new crops and new uses, ASHS, Press, Alexandria*, 509–513.
[3] Welsh J. and McClelland M. (1990) *Nucleic Acids Res*., 18: 7213–7218.
[4] Das M., Bhattacharya S., Basak J. and Pal A. (2007) *Biologia Plantarum*, 51(4): 667-672.
[5] Sneath P.H.A. and Sokal R.R. (1973). *Numerical taxonomy, Freeman and Co, San Francisco.*
[6] Rohlf F.J. (2000). *NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.2., Exeter Software, Setauket - New York.*
[7] Nei M. and Li W. (1979). *Proc. Nat. Acad. Sci., USA*, 79: 5269-5273.
[8] Nayak S., Rout G.R. and Das P. (2003) *Plant Soil Environ*., 49(1): 24–28.
[9] Raizada M.B. and Chatterjee R.N. (1956). *Indian Forester*, 82: 215-218.
[10] Bhandari M.S., Kaushal R., Banik R.L. and Tewari S.K. (2015) *Indian Forester*, 141(3): 265-274.
[11] Dasgupta T. and Das P.K. (1991) *Indian J. Agril. Res*., 25(1): 7-13.
[12] Yan W. and Kang M.S. (2003) *GGE Biplot Analysis: A Graphical tool for Breeders. Geneticist and Agronomist, University of Guelph, Ontario-Canada.*
[13] Generoso A.L., Santos J.O., Carvalho V.S., Sacoman N.N. and Rodrigues R. (2016) *Rev. Ciênc. Agronômica, Agricultural Engineering*, 47(1): 1-9.
[14] Gamble J.S. (1896). *Bambuseae of British India - Ann. Roy. Bot. Garden, Calcutta.*
[15] Brown-Guedira G.L., Thompson J.A., Nelson R.L. and Warburton M.L. (2000). *Crop Sci*., 40: 815-823.
[16] Ghosh S., Somkuwar B., Mandi S.S. and Talukdar, N.C. (2012) *Asian Journal of Plant Science and Research*, 2(4): 478-485.
[17] Wang X., Ye X., Zhao L., Dezhu Li., Guo Z. and Zhuang, H. (2017) *Sci Rep*., 7: 11546.
[18] Yang H.Q., Yang J.B., Peng Z.H., Gao J., Yang Y.M., Peng S., Li D.Z. (2008) *Molecular Phylogenetics and Evolution*, 48: 809–824.
[19] Eevera T., Rajandran, K., Saradha S., Lashmi A. (2008) *Tree and Forestry Science and Biotechnology*, 2(1): 54-56
[20] Fisher A.E., Clark L.G. and Kelchner S.A. (2014) *Systematic Botany*, 39(3): 829-844.
[21] Clark L.G., Dransfield S., Triplett J., and Sa´Nchez-Ken J.G. (2007) *Aliso: A Journal of Systematic and Evolutionary Botany*, 23: 315–332
[22] Triplett J.K., Oltrogge K.A. and Clark L.G. (2010) *American Journal of Botany*, 97(3): 471–492.
[23] Kobayashi M. and Furumoto R. (2004) *Journal of Phytogeography and Taxonomy*, 52: 1-24.
[24] Danquah Owusu E., Akromah R., Quashie-Sam S.J., Oduro W., Falk D., Thevathasan N.V. and Gordon A.M. (2012) *Agroforest Syst*., 86(3), 443–450.