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Phylogenetic Proximity among Twenty Accessions of *Dendrocalamus strictus* Unfolded by Protein Profiling and Culm Sheath Descriptors

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Abstract

The wide distribution and adaptability of *D. strictus* encompasses great diversity which is expected to be reflected in its genetic constitution, hence identification and characterization of its genetic resources assumes great significance. The present work was carried to assess diversity in *D. strictus* germplasm through Culm sheath characteristics and Protein profiling. Euclidean method linkage ward cluster was carried on culm sheath descriptors. Three clusters were obtained with first cluster further segregating into two sub clusters, with sub cluster I comprising A1, A20 whereas sub cluster II including A5, A10, A40, A38, A19, A8, A36, A7, A11, A88 and A13. The second cluster comprised of accession A16, A23, A17, 18, A28, A32, underlining a fair degree of phylogenetic relatedness. A35, an outlier in the dendrogram parted separately, featuring an apparent remoteness from rest of the accessions. Cluster analysis for protein profiling of the *D. strictus* genotypes employing UPGMA analysis led to segregation of twenty accessions and their ultimate grouping into four clusters which were further subdivided into subclusters. From Jaccards similarity coefficient, the accessions in cluster I showed high genetic similarity. Cluster IV contained singular accession A36 (Hoshiarpur II) which was found to be genetically divergent from the rest of the accessions. Genetic diversity ranged from 89% (0.89) to 11% (0.11) representing a broad base which warrants further documentation, characterization and conservation for *D. strictus* improvement.

Keywords: Diversity; *D. strictus*; Protein profiling; Culm sheath descriptors

Introduction

Culm sheath is indispensable diagnostic tool in identification of bamboos and could be used to prepare a key for identification of standing culms of selected species. Banik [1] observed the presence or absence of culm sheath, nodal rootings and branching pattern as diagnostic for determining the age of culm from one to four years. Das et al. [2] extended his work on *Bambusa tulda* to describe the species for morphological characters (vegetative and reproductive) in order to enable the species identification at various stages of life cycle. He studied 32 morphological characters (15 culm and 17 culm sheath) along with detailed inflorescence and floral characters. The description of culm, culm sheath, inflorescence and floral morphology were in agreement with the prior taxonomic description. Gamble [3] was the first to extensively use various culm sheath features at species level and were later employed at generic level too Nakai [4] since they are often very informative even for higher taxonomic rank such as subfamily for instance *Bambuseae* and *Olyraceae* are clearly differentiated on the basis of presence or absence of the abaxial ligule. Raizada et al. [5] studied the morphology of culm sheaths to identify different species of bamboo, they found culm sheath diagnostic in identification of bamboos. Bahadur [6] studied culm sheaths and juvenile vegetative buds and prepared a key for identification of standing culms of selected species. Prakash [7] noted the exploitability of SDS-PAGE as a highly useful tool for discerning various micro evolutionary processes. Biswas et al. [8] carried out studies on *Dendrocalamus strictus* assaying ten enzyme systems by horizontal starch gel electrophoresis through the seedlings raised from the seeds collected from gregarious flowering in Doon valley in year 1994. Ten systems used were PGI, DIA, AAT, GOT, LAP, EST, GDH, IDH, MDH and 6PGDH. Leaf and rhizomatous tissues showed common features in banding patterns under PGI, LAP and 6 PGDH. Exploitation of morphological studies coupled with sophisticated technique like protein profiling for prediction of heterotic performance could enhance opportunities to obtain superior hybrid due to a higher probability of unrelated genotypes contributing unique desirable alleles at different loci.

Materials and Methods

Twenty different accessions of *D. strictus* were selected from Dendrosetum at Forest Research Institute (FRI) Dehradun, harboring *D. strictus* clones collected from different eco-geographical zones of India in the year 2008 under the project "Bamboo improvement for rural and tribal communities, integrating new technologies" funded by National Bamboo Mission, New Delhi (Table 1).

Taxonomic study

Each accession was considered as separate independent operational taxonomic unit (OTU). A comparative account of key culm sheath descriptors were used to evaluate phylogenetic relationships among 20 bamboo species (OTU's) which are as follows:

Length / breadth at base. Ciliate margin (absent=0, present=1) Pubescent adaxial hair (absent=0, present=1), Pubescent abaxial hair (absent=0, present=1) Hair color (none=0, golden brown=1, brown=2, dark brown=3) Number or density of hairs (absent=0, scanty=1, profuse=2), Shape of blade (triangular=0, acuminate=1), Blade reflexed (absent=0, present=1) Hairy margin on the blade (absent=0, present=1), Ligule margin (0=wavy, 1=dome shaped, 2=indented, 3=slanted or oblique, 4=straight or entire) Hairs on ligule (absent=0, present=1) Auricles (0=absent, 1=present), Variable sheath sizes at different culm heights (0=absent, 1=present).

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Protein profiling

The young foliage of selected twenty accessions was collected, tagged properly in a polybag and stored in ice bucket till it was brought to Plant Physiology lab and stored at -20°C. Protein was extracted from each of the accessions ensued by the SDS-PAGE electrophoresis. Leaves homogenized in 0.1M Tris Hcl buffer (pH 8.3) using pre-chilled mortar and pestle was centrifuged at 10,000 rpm for 30 min to extract Protein. The protein extract was taken in equal volume (1:1 ratio) of SDS sample buffer (Laemmli buffer) containing 0.5M Tris HCl buffer (pH 6.8), 5% Mercaptoethanol, 10% Glycerol, 10% SDS (Sodium dodecyl sulphate) as described by Laemmeli [9].

Statistical analysis

Genetic diversity was analyzed among the accessions through construction of different clusters on the basis of taxonomic traits using Euclidean method, Linkage Ward cluster analysis using Minitab release 11.2. For protein profiling, the scoring of bands was analyzed by binary method of present (1) and absent (0). The data was subjected to cluster analysis in UPGMA (Unweighed pair grouping method) using Jaccard's similarity coefficient for phylogenetic relationship.

Results

Variability in culm sheath characters (Figures 1-4; Tables 2 and 3).

Discussion

The culm sheath is the most important diagnostic feature for taxonomic characterization. The culm sheath was found to vary immensely among the accessions. The genotypes displaying maximum culm sheath area were 11, 28, 35 and 88. On the basis of non-parametric traits, the accessions separated into three clusters with

Figure 1: Diversity in Culm sheath, variation in ligule characteristics, color, texture, hair +/- (color).

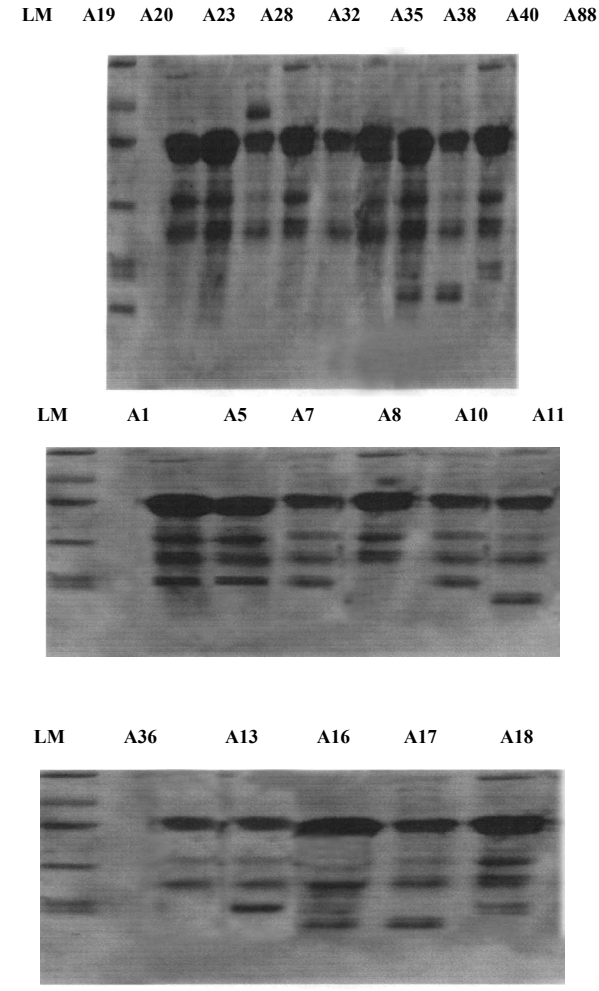

Figure 2: SDS PAGE protein banding pattern. Variability in banks (thickness, tightness, visibility).

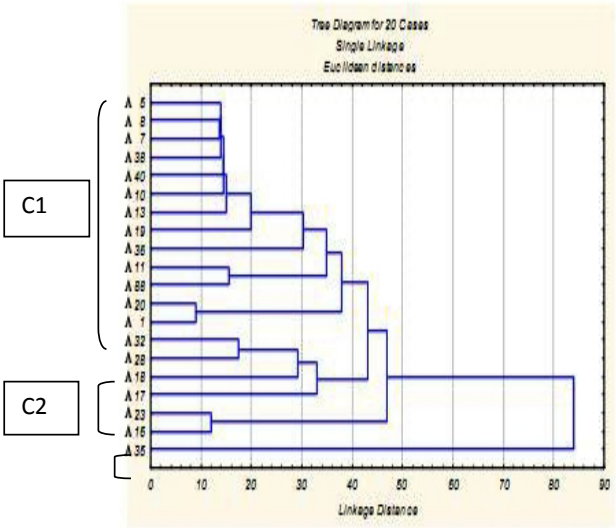

Figure 3: UPGMA based on culm sheath descriptors.

Serial. No	Accession code	Provenances	Latitude (°N)	Longitude (°E)	Altitutde	
1	A-1	Biyasi	30°44′	78°27′	1352	Uttrakhand, India
2	A-5	Devprayag	30°15′	78°6′	830	
3	A-7	Mansadevi	26°92′	78°15′	444	
4	A-8	Haridwar	26°96′	78°16′	315	
5	A-10	Shyampur	26°74′	78°11′	310	
6	A-11	Sahaspur	26°73′	78°05′	311	
7	A-13	Kalsi	30°32′	78°03′	510	
8	A-16	Bhogpur	30°1′	77°28′	255	Harayana
9	A-17	Pinjore	30°79′	76°91′	550	
10	A-18	Thadugarh	30°73′	76°78′	225	
11	A-19	Seonthi	30°2′	74°23′	250	
12	A-20	Kurukshetra	29°6′	77°04′	222	
13	A-23	Hissar	29.15′	75.71′	210	
14	A-28	Ropar	30°96′	76°53′	262	Punjab
15	A-32	Kahanpur,	26°46′	80°33′	228	
16	A-35	Hoshiarpur I	31°53′	75°92′	296	
17	A-36	Hoshiarpur II	31°52′	75°90′	294	
18	A-38	Dasuya	31°82′	75°66′	240	
19	A-40	Jhelwa	31°5′	75°6′	250	
20	A-88	Andhra pradesh	17°36′	78°47	536	AP

Table 1: Geographical information of the accessions taken for study.

Cluster No	Accessions		Cluster No.	Sub-Cluster	Accessions
Cluster I	A5, A8, A7, A38, A40, A10, A13, A19, A35, A11, A88, A20, A1	Compare and contrast between the two approaches with respect to clustering pattern	**Cluster I**	Sub-cluster 1	A19, A35, A28, A20, A8, A18
Cluster II	A32, A28, A18,			Sub-cluster 2	A5, A10, A7, A1
Cluster III	A23, A17			Sub-cluster 3	A38
Cluster IV	A35			Sub-cluster 4	A16, A88, A11.
			Cluster II	Sub-cluster 1	A32
			Cluster III	Sub-cluster 1	A17, A23, A40
			Cluster IV	Sub-cluster 1	A36

Table 2: Cluster analysis employing using upgma and jaccards similarity coefficient.

S. No	Genotype	Molecular classification	Taxonomic classification
1	A1, A20, A5, A10, A38, A19, A8 A11, A88, A13, A7	Same cluster (Cluster I)	Same cluster (Cluster I)
2	A36, A40	Different cluster	Same cluster (Cluster I)
3	A18, A35, A28, A16	Same cluster (Cluster I)	Different clusters
4	A32	Same cluster (Cluster II)	Same cluster (Cluster II)
5	A23*, A17**	Different cluster (Cluster III)	Same cluster (ClusterII)

A23*and A17** were found in cluster II on the basis of taxonomic classification and cluster III in phenetic tree, although segregating in different clusters using two different approaches, they always remain together which indicates a possible phylogentic relationship. Over all 60-70% of consonance is observed in results based on clustering pattern

Table 3: Dendrogram analysis of taxonomic and molecular approach.

majority of accessions (A1, A20 A5, A10, A40, A38, A19, A8, A36, A7, A11, A88, A13.) falling into first cluster and (A16, A23, A17, 18, A28, A32) into second cluster respectively. The third cluster with a single accession A35 can be seen in Figure 3. Vegetative characters mainly describing culm and culm sheath were widely used for bamboo species determination by Ohrenberger et al. [10]. The maximum intercluster distance from cluster centroids is 220.35 units, specifying that the accessions in cluster I and cluster IV were more divergent.

Owing to long sexual cycles and unavailability of reproductive material in bamboos, vegetative characters (culm and culm sheath) are normally used as a diagnostic tool for identification. However, Wu [11] remarked the unreliability of such methods primarily due to influencing environment. The shortcoming of vegetative characters to distinguish closely allied species was also noted by Das et al. in bamboos. The accessions of different eco-geographical regions showing altogether different taxonomic traits may not be genetically diverse. The reflection of such divergence may be due to interaction of environment and genetic constitution, thus it is necessary to confirm the diversity at the molecular level. Protein profile/fingerprinting method has been useful in addressing the question on genetic nature of population and their conservation Moran et al. [12]. unlike morphological and phenological traits that are often polygenic and influenced by environmental conditions.

The present study revealed significant protein polymorphisms among the twenty accessions. Seven reproducible prominent bands with range of 14.3 kD to 94.7 kD were obtained. The highest numbers

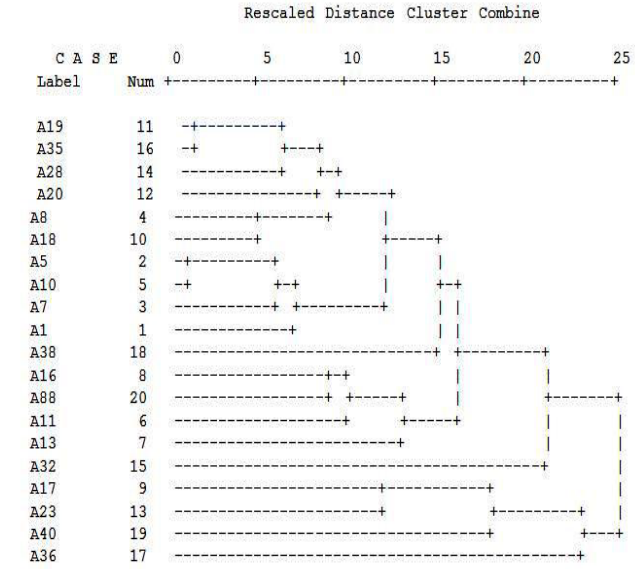

Figure 4: Phenetic tree constructed from similarity matrix of protein banding pattern.

of bands were observed in genotypes A8 and A18, whereas lowest number of bands was observed in genotype A36.

Protein profile analysis revealed that the genetic similarity varied from a high 89% to as low as 11% (0.11). Such a high range of similarity coefficient values suggest that *D. strictus* germplasm collection represents a high variation and a broad genetic base which needs to be documented, characterized, conserved and used for its *D. strictus* improvement. Also accessions A32 from Kahanpur and A36 from Hoshiarpur II split into separate clusters appearing as genetically divergent population and hence could be used as outlier population for further breeding and tree improvement work. The findings were in harmony with the Das et al. [2] who also obtained a high range of similarity coefficient value 50 to 90% in various bamboos species.

Conclusion

Significant degree of variation was observed with respect to culm sheath characteristics. Also a broad genetic base (0.89-0.11) was revealed using similarity matrix. Accession A35 and A36 (notably, both accessed from Punjab) segregated from the rest of the accessions using taxonomic and biochemical approach. 60-70% consistency in results adopting the two approaches infers complementarities into play, thus exploitation of vegetative traits coupled with sophisticated technique like protein fingerprinting for prediction of heterotic performance could enhance opportunities to obtain superior hybrid due to a higher probability of unrelated genotypes contributing unique desirable alleles at different loci.

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