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# Genetic diversity among economically important bamboo species of India as revealed by ISSR markers

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**Abstract**

Bamboo taxonomy rests largely on vegetative and culm-sheath characters, which are diagnostic on mature culms but show marked phenotypic plasticity across sites and culm ages, while reproductive characters are seldom available because flowering is gregarious, unpredictable and separated by cycles of 3–120 years. Molecular markers therefore provide a complementary rather than a substitute route to characterisation**.** In the present study, an inter-simple sequence repeat (ISSR) marker system employing 21 primers was used to study the genetic relationships among 15 bamboo accessions representing 13 species in four genera, collected from different regions of India. The 21 primers amplified 316 scoreable bands, of which six primers yielded 15 or more bands each; the maximum was recorded for UBC826 (20 bands), followed by UBC827 (19). Polymorphism reached 99.7 %, and polymorphism information content averaged 0.312 per primer against a theoretical maximum of 0.5 for a dominant marker. Similarity coefficients based on simple matching varied between 0.52 and 0.96. The phylogenetic tree based on the genetic similarity matrix, constructed using the unweighted pair group method with arithmetic average (UPGMA) algorithm, resolved the 15 accessions into seven clusters. The highest similarity (0.96) was recorded between *Arundinaria falcata* and the unidentified hill bamboo accession, supporting its provisional placement in *Arundinaria*, followed by *Bambusa vulgaris* var. yellow and var. wamin (0.81). Lowest genetic similarity was observed between *B. tulda* and *D. strictus* (0.50), followed by *B. tulda* and *B. balcooa* (0.57). Clustering was broadly, though not entirely, congruent with the conventional classification, the six *Bambusa* accessions other than *B. tulda* forming a single group. Because ISSR markers are dominant and woody bamboos are predominantly polyploid, the results are interpreted as phenetic similarity among clonal accessions rather than as allele-frequency-based population diversity. Results suggest the possibility of selection among, and hybridisation between, accessions drawn from divergent clusters.

**Keywords:** Bamboo, Simple matching coefficient, Genetic diversity, ISSR marker, UPGMA

Worldwide, about 100 genera and more than 1,642 species of bamboo are recognised (Kumar *et al.*, 2021). In India the bamboo-bearing area is estimated at 1,54,670 km$^2$ (≈15.47 million ha), an increase of 5,227 km$^2$ over the 2021 assessment (ISFR, 2023). Bamboos are among the most widely used forest plants, being extensively utilised in the production of paper, handicrafts, furniture, housing, water pipes and storage tanks, and with growing demand for timber they offer a viable substitute for timber in the country (Kumar *et al.*, 2023). Overexploitation and genetic erosion make it essential not only to collect and conserve bamboo germplasm but also to classify and describe it (Tewari *et al.*, 2019). Genetic diversity, defined as variability within species, between species and of ecosystems (CBD, 1992, Article 2), provides the opportunity for breeders to select improved cultivars with preferred traits, and its characterisation is the link between germplasm conservation and utilisation (Nayak *et al.*, 2003).

Any plant's identifying characteristics depend largely on floral traits, yet flowering in woody bamboos is a major bottleneck for taxonomic work: the reproductive cycle may span 3 to 120 years (Zheng *et al.*, 2020), so floral material is rarely available. Consequently, bamboo

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taxonomy in practice relies on vegetative characters — culm form, branching pattern and, in particular, culm-sheath morphology — which remain the primary diagnostic basis of the group and are reliable when scored on mature culms (Wang *et al.*, 2025). Their limitation is phenotypic plasticity: the same characters vary with site, culm age and management, which constrains their use for infrageneric and infraspecific discrimination and has motivated the parallel development of molecular approaches (Das *et al.*, 2008). Comparatively little taxonomic research has been done on bamboos, and many economically valuable bamboos of Asia and the Pacific remain unidentified. Identification of promising germplasm groups, optimisation of hybridisation and selection, and conservation all depend on knowledge of the genetic diversity available (Nayak *et al.*, 2003). Isozyme and RAPD markers, and later AFLP, have been used to analyse genetic diversity and relationships in several bamboos, including the subtribe Bambusinae (Loh *et al.*, 2000; Nayak *et al.*, 2003).

First, reference genome sequences and validated SSR or SNP panels are unavailable for most Indian bamboo taxa; ISSR requires no prior sequence information and can therefore be applied directly to uncharacterised germplasm. Second, ISSR primers are 16–25 nucleotides long and are annealed at 42–57 °C, against 10-mer primers annealed near 36 °C for RAPD, which addresses the poor reproducibility that is the principal weakness of RAPD (Das *et al.*, 2008). Third, ISSR targets the hypervariable regions flanking microsatellites and so recovers a higher number of polymorphic fragments per primer than RAPD at comparable cost. Fourth, AFLP, although highly reproducible, requires restriction digestion, adaptor ligation and selective amplification, and is costly and technically demanding on the polysaccharide- and polyphenol-rich DNA typical of bamboo leaf tissue. Fifth, ISSR has an established record of resolving inter- and intra-specific variation in bamboo specifically (Amom *et al.*, 2018; Desai *et al.*, 2015; Goyal and Sen, 2015; Tikendra *et al.*, 2023). Its recognised limitation is equally relevant here: ISSR is a dominant marker scored as band presence or absence, and woody bamboos are predominantly polyploid, so allele dosage cannot be resolved and heterozygotes cannot be distinguished from dominant homozygotes. Results are accordingly interpreted as phenetic similarity between accessions and not as allele-frequency-based population genetic parameters. The objectives of the present research were to test the usefulness of ISSR markers for distinguishing bamboo accessions collected from different parts of India, to compare the resulting groupings with the conventional classification, and to obtain molecular evidence bearing on the identity of one unidentified hill bamboo accession.

**Plant material and germplasm source**

Fifteen bamboo accessions, representing 13 species in four genera (*Bambusa*, *Dendrocalamus*, *Himalayacalamus* and *Arundinaria*), were used (**Table 1**). Twelve accessions are maintained in the field gene bank of the Agroforestry Research Centre (AFRC), G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, and the three hill bamboo accessions in the Himalayan Botanical Garden, Nainital, Uttarakhand. Fresh, fully expanded leaves were collected from three mature clumps per accession during 2022–23. Voucher specimens were deposited and accession numbers assigned as listed in Table 1. All laboratory work was carried out in the Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar.

**DNA extraction**

Genomic DNA was isolated from leaf tissue by the CTAB method of Doyle and Doyle (1990) without modification. DNA quantity and purity were assessed spectrophotometrically at 260 and 280 nm, and integrity was checked by electrophoresis in 1.0 % agarose. Working dilutions of 50 ng $\mu L^{-1}$ were prepared and stored at −20 °C.

**ISSR amplification**

Forty ISSR primers were screened; 21 produced distinct, reproducible and scoreable bands and were retained for analysis **(Table 2**). The remaining 19 primers either failed to amplify or gave weak, smeared or non-reproducible profiles and were discarded. Reactions of 12.82 µL contained 20 ng genomic DNA, 2.5 µL 10X PCR buffer with 15 mM $MgCl_2$, 0.5 µL dNTPs, 1 U Taq polymerase and 20 ng primer. Cycling comprised initial denaturation at 94 °C for 4 min., followed by 45 cycles of 94 °C for 1 min., primer-specific annealing (Table 2) for 1 min. and 72 °C for 2 min., with a final extension at 72 °C for 10 min. Products were resolved on 2 % (w/v) agarose in 0.5X TBE, stained with ethidium bromide and documented on a gel documentation system (Syngene, UK). Fragment sizes were estimated against a 1 kb ladder. Amplification with each primer was repeated to confirm reproducibility.

**Scoring and data analysis**

Clearly resolved bands were scored as present (1) or absent (0) to generate a binary matrix. Primer–accession combinations that failed to amplify or could not be scored reliably were recorded as missing rather than as band-absent, and were excluded pairwise from all subsequent calculations. Pairwise genetic similarity was computed with the simple matching coefficient (Sokal and Michener, 1958) in the SIMQUAL module of NTSYS-pc v2.02e (Rohlf, 1993), and a dendrogram was constructed by UPGMA using the SAHN and TREE modules. Goodness of fit between the dendrogram and the similarity matrix was assessed by the cophenetic correlation coefficient. Polymorphism information content was calculated for each band as $PIC = 1 - p^2 - q^2$ (Anderson *et al.*, 1993), where p is the frequency of band presence and $q = 1 - p$ among the accessions scored for that band, and averaged over the polymorphic bands of each primer. Resolving power was calculated following Prevost and Wilkinson

**Table 1. List of bamboo accessions used for ISSR marker analysis**

| Accession code | Species | Collection site | District / State | GPS coordinates | Institutional code no. | Germplasm maintained at |
|---|---|---|---|---|---|---|
| P1 | *Dendrocalamus strictus* | Kotdwar | Tehri Garhwal, Uttarakhand | GPS | **Institutional code no.** | AFRC, Pantnagar |
| P2 | *Bambusa balcooa* | Gadarpur | U. S. Nagar, Uttarakhand | 29°01′22.5″ N, 79°29′16.3″ E | AFRC DS01 | AFRC, Pantnagar |
| P3 | *Bambusa bambos* | Itanagar | Papum Pare, Arunachal Pradesh | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BBL01 | AFRC, Pantnagar |
| P4 | *Dendrocalamus asper* | Itanagar | Papum Pare, Arunachal Pradesh | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BBS01 | AFRC, Pantnagar |
| P5 | *Bambusa nutans* | Peherwin | Bilaspur, Himachal Pradesh | 29°01′22.5″ N, 79°29′16.3″ E | AFRC DA01 | AFRC, Pantnagar |
| P6 | *Dendrocalamus hamiltonii* | Palampur | Kangra, Himachal Pradesh | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BN01 | AFRC, Pantnagar |
| P7 | *Bambusa vulgaris var. green* | FRI, Dehradun | Dehradun, Uttarakhand | 29°01′22.5″ N, 79°29′16.3″ E | AFRC DH01 | AFRC, Pantnagar |
| P8 | *Bambusa vulgaris var. yellow* | FRI, Dehradun | Dehradun, Uttarakhand | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BVG 01 | AFRC, Pantnagar |
| P9 | *Bambusa vulgaris var. wamin* | FRI, Dehradun | Dehradun, Uttarakhand | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BVY01 | AFRC, Pantnagar |
| P10 | *Dendrocalamus giganteus* | Forest nursery, Lalkuan | Nainital, Uttarakhand | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BVW 01 | AFRC, Pantnagar |
| P11 | *Bambusa multiplex* | FRI, Dehradun | Dehradun, Uttarakhand | 29°01′22.5″ N, 79°29′16.3″ E | AFRC DG01 | AFRC, Pantnagar |
| P12 | *Bambusa tulda* | KFRI, Peechi | Thrissur, Kerala | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BM 01 | AFRC, Pantnagar |
| P13 | *Himalayacalamus falconeri* | Munsyari | Pithoragarh, Uttarakhand | 29°01′22.5″ N, 79°29′16.3″ E | AFRC BT 01 | Himalayan Botanical Garden, Nainital |
| P14 | *Arundinaria falcata* | Nainital | Nainital, Uttarakhand | 29.38° N, 79.45° E | HBG-HF-01 | Himalayan Botanical Garden, Nainital |
| P15 | *Arundinaria sp. (unidentified)* | Nainital | Nainital, Uttarakhand | 29.38° N, 79.45° E | HBG-AF-01 | Himalayan Botanical Garden, Nainital |

(1999) and the marker index as the product of mean PIC and the effective multiplex ratio (Powell *et al.*, 1996).

The 15 accessions studied, their collection sites and the institutions maintaining them are listed in Table 1. They represent 13 species distributed across four genera, six of the accessions belonging to *Bambusa*, four to *Dendrocalamus*, two to *Arundinaria*, one to *Himalayacalamus*, together with three varieties of *B. vulgaris* (green, yellow and wamin) that are treated here as separate accessions of a single species.

Not every primer could be scored for every accession. Of the 315 primer–accession combinations, 45 (14.3 %) failed to amplify or gave profiles that could not be scored reliably, so 644 of the 4,740 cells of the binary matrix (13.6 %) are missing rather than band-absent. The shortfall was not evenly distributed: *Dendrocalamus strictus* (P1) was scored for only 7 of the 21 primers, giving 90 of the 316 bands, while *D. hamiltonii* (P6) and *Arundinaria falcata* (P14) were scored completely. All similarity coefficients were therefore computed on pairwise-complete data, and the number of bands shared between any two accessions ranges from 32 (*D. strictus* with *B. vulgaris* var. wamin) to 316. Comparisons involving *D. strictus* rest on a substantially smaller evidence base than the remainder and are correspondingly less precise. The 21 primers retained after screening, their sequences and annealing temperatures are given in **Table 2**. Sixteen are UBC-series and five ISD-series primers, based on di- and trinucleotide repeat motifs anchored at the 3′ end.

The 21 primers amplified a total of 316 bands. Six primers amplified 15 or more bands each. The maximum number

**Table 2. List of ISSR primers used for molecular diversity analysis**

| S. No. | Primer | Sequence (5'→3') | Annealing temp. (°C) |
|---|---|---|---|
| 1 | UBC 824 | TCTCTCTCTCTCTCTCG | 53.4 |
| 2 | UBC 834 | AGAGAGAGAGAGAGAG(CT)T | 53.1 |
| 3 | UBC 855 | ACACACACACACACAC(CT)T | 52.0 |
| 4 | ISD4 | GTGTGTGTGTGTCG | 47.0 |
| 5 | ISD13 | ATGATGATGATGATGATG | 46.2 |
| 6 | ISD16 | AGAGAGAGAGAGAGAGC | 50.5 |
| 7 | ISD20 | GAGAGAGAGAGAGAGACG | 52.5 |
| 8 | ISD28 | AGAGAGAGAGAGAGAGTA | 47.6 |
| 9 | ISD32 | AGAGAGAGAGAGAGAGAGAGT | 53.4 |
| 10 | ISD40 | TATGCACACTGTGTGTGTGTGTGT | 56.6 |
| 11 | UBC814 | CTCTCTCTCTCTCTCTA | 45.0 |
| 12 | UBC820 | GTGTGTGTGTGTGTGTC | 42.0 |
| 13 | UBC822 | TCTCTCTCTCTCTCTCA | 50.5 |
| 14 | UBC823 | TCTCTCTCTCTCTCTCC | 46.0 |
| 15 | UBC827 | ACACACACACACACACG | 54.0 |
| 16 | UBC809 | AGAGAGAGAGAGAGAGG | 48.2 |
| 17 | UBC821 | GTGTGTGTGTGTGTGTT | 50.3 |
| 18 | UBC810 | GAGAGAGAGAGAGAGAT | 47.0 |
| 19 | UBC826 | ACACACACACACACACC | 53.0 |
| 20 | UBC828 | TGTGTGTGTGTGTGTGC | 53.4 |
| 21 | UBC840 | GAGAGAGAGAGAGAGAYT | 52.0 |

of bands was amplified by UBC826 (20), followed by UBC827 (19), UBC810 and ISD-40 (18 each), UBC822 and UBC823 (17 each), while UBC814 (9) and ISD-28 (7) amplified the fewest. Fragment sizes ranged from 100 to 1,480 bp (**Table 3**). Representative banding profiles for four primers are shown in **Fig. 1**. These results indicate that di-nucleotide-repeat primers anchored with a single selective base were the most productive in this material, consistent with earlier reports in bamboo. The present findings agree closely with Amom *et al.* (2018), who used 10 ISSR primers on 15 bamboos of North-East India and reported UBC 810, 820, 822, 823, 824, 827 and 828 as informative; and with Desai *et al.* (2015), who screened 63 ISSR primers on 13 Indian bamboo accessions and identified ISD-40 and ISD-28 as useful. Both sets of primers were among those used here.

Polymorphism was almost complete: 315 of the 316 bands (99.7 %) were polymorphic across the accessions scored, and 20 of the 21 primers gave 100 % polymorphic bands, the exception being ISD28 (six of seven bands, 85.7 %). PIC calculated per band and averaged over each primer ranged from 0.220 (UBC 855) to 0.396 (ISD20), with a mean of 0.312 (**Table 3**). Since ISSR bands are dominant and scored as presence or absence, PIC for a single band is bounded above by 0.5, so the observed mean represents approximately 62 % of the attainable maximum and indicates that the primer set was informative for this material. Resolving power averaged 6.60 per primer and was highest for ISD32 (9.50), ISD20 (9.43) and UBC 826 (9.23). The marker index averaged 4.66 and was highest for UBC 826 (6.49), reflecting its combination of the largest number of bands (20) with above-average PIC. ISD28 was the least informative primer on every measure (Rp 2.40, MI 1.54) and could be dropped from future work on this germplasm without material loss.

The similarity matrix based on the simple matching coefficient is given in **Table 4**. Similarity coefficients ranged from 0.52 to 0.96, with a mean of 0.66. The highest value (0.96) was found between *A. falcata* and the unidentified *Arundinaria* accession, followed by *B. vulgaris* var. yellow and *B. vulgaris* var. wamin (0.81), *H. falconeri* and *A. falcata* (0.76), *B. nutans* and *B. vulgaris* var. wamin (0.76), and *D. strictus* and *B. balcooa* (0.76). The lowest similarity was recorded between *B. tulda* and *D. strictus* (0.50), followed by *B. tulda* and *B. balcooa* (0.57), and *B. vulgaris* var. green and *Arundinaria* sp. (0.58). This range indicates moderate to high divergence among the accessions and, since it spans both closely related and clearly distinct material, is favourable for conservation and for breeding, where crosses between accessions from divergent clusters may generate transgressive segregants.

**Table 3. Polymorphism features of the 21 ISSR primers across 15 bamboo accessions**

| S. No. | Primer | Size range (bp) | Total bands | Polymorphic bands | Polymorphism (%) | PIC | Rp | MI |
|---|---|---|---|---|---|---|---|---|
| 1 | UBC 824 | 100–1000 | 16 | 16 | 100.0 | 0.226 | 4.46 | 3.62 |
| 2 | UBC 834 | 130–1000 | 12 | 12 | 100.0 | 0.333 | 5.85 | 4.00 |
| 3 | UBC 855 | 295–1390 | 15 | 15 | 100.0 | 0.220 | 4.00 | 3.31 |
| 4 | ISD4 | 315–1150 | 12 | 12 | 100.0 | 0.347 | 6.15 | 4.17 |
| 5 | ISD13 | 310–1250 | 14 | 14 | 100.0 | 0.297 | 5.83 | 4.15 |
| 6 | ISD16 | 250–1200 | 15 | 15 | 100.0 | 0.368 | 8.31 | 5.51 |
| 7 | ISD20 | 190–1300 | 15 | 15 | 100.0 | 0.396 | 9.43 | 5.94 |
| 8 | ISD28 | 390–1000 | 7 | 6 | 85.7 | 0.300 | 2.40 | 1.54 |
| 9 | ISD32 | 230–710 | 16 | 16 | 100.0 | 0.386 | 9.50 | 6.18 |
| 10 | ISD40 | 170–1480 | 18 | 18 | 100.0 | 0.334 | 8.92 | 6.01 |
| 11 | UBC814 | 310–805 | 9 | 9 | 100.0 | 0.308 | 3.85 | 2.77 |
| 12 | UBC820 | 410–1370 | 15 | 15 | 100.0 | 0.322 | 6.91 | 4.83 |
| 13 | UBC822 | 188–1350 | 17 | 17 | 100.0 | 0.330 | 8.31 | 5.61 |
| 14 | UBC823 | 285–1100 | 17 | 17 | 100.0 | 0.348 | 8.43 | 5.91 |
| 15 | UBC827 | 330–1316 | 19 | 19 | 100.0 | 0.283 | 7.00 | 5.38 |
| 16 | UBC809 | 150–550 | 15 | 15 | 100.0 | 0.298 | 6.00 | 4.47 |
| 17 | UBC821 | 356–1250 | 14 | 14 | 100.0 | 0.238 | 4.15 | 3.34 |
| 18 | UBC810 | 300–1450 | 17 | 17 | 100.0 | 0.292 | 6.62 | 4.97 |
| 19 | UBC826 | 152–890 | 20 | 20 | 100.0 | 0.324 | 9.23 | 6.49 |
| 20 | UBC828 | 193–812 | 15 | 15 | 100.0 | 0.314 | 6.71 | 4.70 |
| 21 | UBC840 | 180–880 | 18 | 18 | 100.0 | 0.281 | 6.57 | 5.06 |
| **Total / Mean** | | **100–1480** | **316** | **315** | **99.7** | **0.312** | **6.60** | **4.66** |

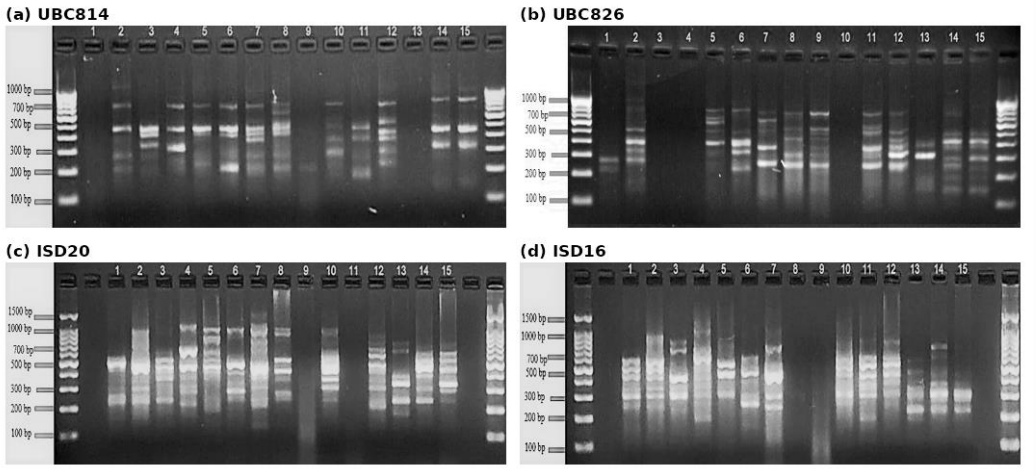


**Fig. 1. ISSR banding profiles of 15 bamboo accessions (lanes 1-15) generated by primers (a) UBC 814, (b) UBC 826, (c) ISD20 and (d) ISD16**

UPGMA clustering resolved the 15 accessions into seven clusters at a cut-off of 0.68 (**Fig. 2**), with a cophenetic correlation of 0.79 between the dendrogram and the similarity matrix. Cluster V was the largest, containing six accessions, followed by Cluster II (three) and Cluster I (two); Clusters III, IV, VI and VII each contained a single accession. Six of the seven *Bambusa* accessions — *B. bambos*, *B. nutans*, *B. vulgaris* var. yellow, var. wamin and var. green, and *B. multiplex* — grouped together in Cluster V, supporting their placement in a single genus under the conventional system. *B. tulda* was separated from this group, indicating lower genetic affinity with the remaining *Bambusa* accessions. This contrasts with Loh *et al.* (2000), who reported close affinity between

**Table 4. Simple matching similarity coefficients between pairs of bamboo accessions**

| | Species | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 | P13 | P14 | P15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P1 | *D. strictus* | 1 | | | | | | | | | | | | | | |
| P2 | *B. balcooa* | 0.76 | 1 | | | | | | | | | | | | | |
| P3 | *B. bambos* | 0.66 | 0.64 | 1 | | | | | | | | | | | | |
| P4 | *D. asper* | 0.66 | 0.64 | 0.67 | 1 | | | | | | | | | | | |
| P5 | *B. nutans* | 0.63 | 0.62 | 0.75 | 0.68 | 1 | | | | | | | | | | |
| P6 | *D. hamiltonii* | 0.63 | 0.64 | 0.62 | 0.64 | 0.65 | 1 | | | | | | | | | |
| P7 | *B. vulgaris var. green* | 0.61 | 0.63 | 0.67 | 0.62 | 0.72 | 0.68 | 1 | | | | | | | | |
| P8 | *B. vulgaris var. yellow* | 0.66 | 0.62 | 0.71 | 0.66 | 0.72 | 0.65 | 0.73 | 1 | | | | | | | |
| P9 | *B. vulgaris var. wamin* | 0.63 | 0.65 | 0.70 | 0.70 | 0.76 | 0.67 | 0.68 | 0.81 | 1 | | | | | | |
| P10 | *D. giganteus* | 0.67 | 0.61 | 0.66 | 0.63 | 0.69 | 0.63 | 0.68 | 0.64 | 0.69 | 1 | | | | | |
| P11 | *B. multiplex* | 0.70 | 0.65 | 0.72 | 0.67 | 0.72 | 0.64 | 0.69 | 0.64 | 0.70 | 0.67 | 1 | | | | |
| P12 | *B. tulda* | 0.50 | 0.57 | 0.62 | 0.62 | 0.69 | 0.63 | 0.65 | 0.68 | 0.69 | 0.67 | 0.68 | 1 | | | |
| P13 | *H. falconeri* | 0.72 | 0.61 | 0.69 | 0.65 | 0.69 | 0.68 | 0.64 | 0.67 | 0.73 | 0.67 | 0.68 | 0.65 | 1 | | |
| P14 | *A. falcata* | 0.72 | 0.59 | 0.69 | 0.61 | 0.66 | 0.62 | 0.60 | 0.63 | 0.65 | 0.66 | 0.66 | 0.62 | 0.76 | 1 | |
| P15 | *Arundinaria sp.* | 0.74 | 0.61 | 0.70 | 0.62 | 0.67 | 0.62 | 0.58 | 0.61 | 0.65 | 0.65 | 0.67 | 0.63 | 0.74 | 0.96 | 1 |

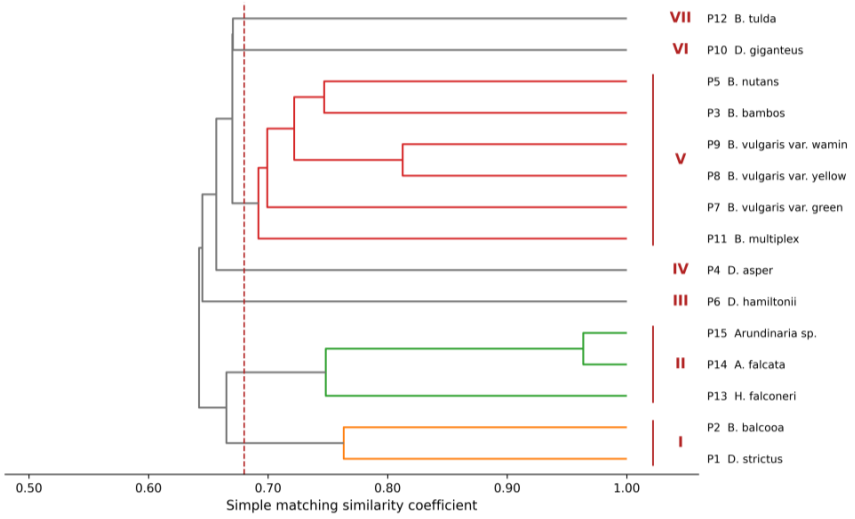


**Fig. 2. UPGMA dendrogram showing genetic relationships among 15 bamboo accessions based on ISSR markers**

*B. tulda* and *B. vulgaris* using AFLP, but agrees with Desai *et al.* (2015) and Amom *et al.* (2018), who both recorded low similarity between these taxa using ISSR.

Accessions collected from widely separated regions were grouped together: *B. bambos* from Itanagar (Arunachal Pradesh) and *B. nutans* from Peherwin, Bilaspur (Himachal Pradesh) clustered with the *B. vulgaris* varieties and *B. multiplex* collected from FRI, Dehradun (Uttarakhand). This indicates that genetic divergence in this material is not a function of geographical separation, in agreement with Gami *et al.* (2015), who reported the same independence in 20 bamboo accessions collected across India. It should be noted, however, that most accessions are maintained ex situ in common plantations, so their present location does not necessarily reflect their origin, and the comparison is between reported provenances rather than between wild populations.

The unidentified hill bamboo accession showed a banding profile closely matching that of *A. falcata* (similarity 0.96) and clustered with it. On this evidence the accession is provisionally assigned to *Arundinaria*. ISSR profiles alone do not constitute a species-level identification, and confirmation by sequencing of standard barcode regions together with examination of culm-sheath characters on mature culms is recommended before the assignment is treated as settled. *A. falcata* and *H. falconeri* were grouped together, consistent with the morphological features they share.Although some disagreement remains, the

relationships recovered by ISSR markers among the 15 accessions from different parts of India were reasonably consistent with the conventional method of classification, as also reported by Goyal and Sen (2015) for bamboos of North Bengal.

The present study confirms the effectiveness of ISSR markers in revealing genetic diversity and resolving relationships among bamboo accessions from different regions of India. Highly polymorphic primers, particularly UBC 826, UBC 827, ISD-40, UBC 822, UBC 823 and UBC 810, proved reliable in detecting variation. Similarity coefficients ranging from 0.50 to 0.96 indicate the presence of both distinct and closely related material, a prerequisite for conservation and genetic improvement. Cluster analysis broadly supported conventional taxonomic classification, particularly within *Bambusa*, while also revealing divergence such as the distinct placement of *B. tulda*. Grouping of accessions from different regions within the same clusters indicates that genetic divergence is independent of geographical origin. The provisional identification of the hill bamboo accession as *Arundinaria* sp. illustrates the value of ISSR profiling in resolving ambiguity among morphologically similar material, subject to confirmation by sequence-based methods. Overall, the variability detected provides a resource for bamboo breeding programmes, in which selection and hybridisation among divergent clusters may yield superior and resilient material. The dominant, biallelic nature of ISSR and the polyploid genomes of woody bamboos limit these inferences to phenetic similarity; codominant or sequence-based markers would be required for allele-frequency-based population genetic analysis.

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