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# Assessment of Genetic Diversity of four montane Bamboo species of Central and North-Western Himalayan region

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# Abstract

Bamboo is a valuable forest resource with substantial ecological and economic significance. As a multipurpose species, it is used for food, fuel, and fodder. Overexploitation of bamboo genetic resources has exerted significant pressure on natural populations, especially affecting dwarf bamboo species in hilly regions. Assessing molecular diversity is critical for the development of effective conservation and breeding approaches. In this research, genomic DNA was isolated using the CTAB method with liquid nitrogen. Forty accessions representing *Thamnocalamus falconeri*, *Arundinaria falcata*, *Sinarundinaria anceps, and Thamnocalamus spathiflora* were analyzed using 42 random primers. Binary (1/0) data facilitated the inference of population parameters, and analysis of molecular variance (AMOVA) was performed to evaluate molecular diversity across all four species. A total of 248 loci were amplified, with an average resolving power (Rp) of 12.97 and a polymorphism information content (PIC) of 0.32. The mean Nei's gene diversity (h) was 0.391, with values ranging from 0.179 to 0.29. The observed number of alleles (na) ranged from 1.473 to 1.716, while the effective number of alleles (ne) ranged from 1.3084 to 1.515 among the four hill bamboo species. Pairwise Nei's genetic distance values ranged from 0.1507 to 0.4663.


# Introduction

Bamboo is a multipurpose species that serves as an integral component of many agroecosystems. Its cultivation is progressively prioritized due to low input requirements and multiple applications, including use as building material, soil erosion prevention, and livestock feed. Bamboo is well-suited for sustainable land use in agroforestry systems, as it prospers on various soil types and requires minimal water. Bamboo leaves are an important component of cattle diets, supporting animal performance throughout the year, particularly during periods of fodder scarcity. The consumption of bamboo leaves enables cows to maintain milk production during the dry season lacking significant effects on yield (Andriarimalala et al., 2019).

Biodiversity refers to the variety within and among living organisms, assemblages, biotic communities, and biotic processes, whether naturally occurring or modified by humans (DeLong, 1996). Increasing population pressure has led to the continuous depletion of natural resources, bringing about notable loss of biological diversity. This loss is irreversible and constitutes major threats to the survival of living organisms on Earth. Consequently, conservation and sustainable management of natural resources are imperative for the protection of forest genetic resources.

Both *in situ* and *ex situ* approaches to genetic conservation can be implemented for the long-term preservation and sustained improvement of genetic resources. Germplasm conservation has exhibited considerable effect on both conservation and improvement programs. Germplasm characterization is a prerequisite for the management and maintenance of genetic resource identity and purity, assuring their effective utilization in future breeding programs.

Marker technologies are used in germplasm acquisition, assessing plant diversity, checking the stability of conserved germplasm, and managing gene banks. Understanding gene flow and how genetic variation is distributed within and between populations is important for conservation, as both influences the genetic structure of populations (Dawson et al., 1997).

Dwarf bamboos are distinct from tropical bamboo species. These dwarf species are extensively utilized for various household and economic purposes, resulting in overexploitation and reduced population viability. Natural regeneration, which mainly occurs through seeds, is problematic because gregarious flowering events cause the death of entire clumps after seeding. Additional factors such as intensive grazing, fire, and agricultural encroachment add further to to the degradation of hill bamboo populations and the reduction of their genetic diversity.

Germplasm characterization and evaluation can be effectively performed using DNA-based marker techniques. The documented morphological variability among hill bamboo populations and individual plants is presumed to be governed by underlying genotypic variation. Quantifying this genetic variation is essential to select appropriate breeding and hybridization strategies. This study aimed to assess the molecular diversity of four hill bamboo species—*A. falconerii*, *A. falcata*, *S. anceps*, and *T. spathiflora* germplasm at Khirsu (Pauri), Uttarakhand, India, established by the Forest Research Institute (FRI), Dehradun. Research Institute (FRI), Dehradun.

# Materials and Methods

### Plant material

The young leaves of selected accessions of Four dwarf bamboo species namely; *Sinarundinaria anceps*, *Arundinaria falcata*, *Thamnocalamus spathiflorus* and *Thamnocalamus falconeri* from Hill bambusetum at Khirsu, Pauri Garhwal ($30^0$10.368’ N latitude, $78^0$ 52.167’ E longitude and 1934 m altitudes above sea level), established in year 2008 by Forest Research Institute, Dehradun, were collected, tagged and brought in ice bucket at Plant Physiology Laboratory, FRI, Dehradun. The collected leaves were kept at -20°C in deep freezer (vest frost DFS 345) for the experiments.

### Plant DNA extraction

Genomic DNA isolation was done by using CTAB method developed by Stange et al. (1998) from the collected leave samples, necessary modifications were done in the standard protocol for extractions of quality genomic DNA. Biophotometer ($A_{260/280}$) for DNA quantification and 1% agarose gel electrophoresis for quality assessment were performed.

**Molecular analysis**: A reaction mixture (25 µl) was prepared containing 10X Taq buffer, 2 mM dNTPs, 2.5 mM $MgCl_2$, Taq Polymerase (5 U/µl), 20 mM primer, and 50 ng/µl genomic DNA. RANDOM-PCR analysis was conducted using a thermal cycler (Techne, FPROG05D) with the following cycling program: initial denaturation at 94°C for 4 minutes, denaturation at 94°C for 1 minute, elongation at 37°C for 1 minute,

and termination at 72°C for 2 minutes, repeated for 45 cycles. A final extension was performed at 72°C for 5 minutes. Amplified PCR products were separated by 1.5% (w/v) agarose gel electrophoresis.

# Observations

### Diversity parameters

For each population (species) the number of observed alleles (na), the effective number of alleles (ne), Nei's gene diversity (h), Shannon's index of genetic variation (I), the number of polymorphic loci and the percentage of polymorphic loci were calculated. The estimates of gene flow (Nm) derived from Gst or Gcs (estimates of genetic differentiation) were based on the allelic frequency data and computed as Nm = 0.5(1 - Gst)/Gst in accordance with Nei (1987). The variance components together with their significance levels for variation among populations and within populations were obtained from the data on 40 genotypes (10 for each species). The analysis was carried out using the Popgen32 software.

### AMOVA analysis

Molecular variance was calculated using analysis of molecular variation (AMOVA) implemented in the GenAlEx (Genetic Analysis in Excel) v. 6.1 package (Peakall, Smouse, 2006).

Table 1
Geographical Details of different accessions of four Ringal species at Hill bambusetum at Khirsu, Pauri Garhwal

| ***A. falcata*** | | |
|---|---|---|
| **Accession. No.** | **ID. No.** | **Place of Collection** |
| A1. | 126 | Hanumanchatti, Uttarkashi |
| A2. | 5 | PithondiKhirsu. Pauri |
| A3. | 10 | Maroda Village, Khirsu, Pauri |
| A4. | 1 | Mandal I, Chamoli. |
| A5. | 151 | Solan, H.P. |
| A6. | 38 | Netwar, Uttarkashi |
| A7. | 58 | Dewal, Chamoli |
| A8. | 3 | Mandal II, Chamoli. |
| A9. | 4 | Mandal III, Chamoli. |
| A10. | 33 | Jarmola, Uttarkashi |
| **T. falconeri** | | |
| B1. | 13 | FRH, Mandal I, Chamoli |
| B2. | 73 | Van I, Bednibugyal, Chamoli |
| B3. | 15 | FRH II, Mandal, Chamoli |
| B4. | 77 | Van II, Bednibugyal, Chamoli |
| B5. | 103 | Munsyari I, Pithoragarh |
| B6. | 16 | Musk deer farm I Chopta, Chamoli |
| B7. | 80 | Van III, Bednibugyal, Chamoli |
| B8. | 17 | Musk deer farm II Chopta, Chamoli |
| B9. | 12 | Musk deer farm III Chopta, Chamoli |
| B10. | 100 | Munsyari II, Pithoragarh |
| **S. anceps** | | |
| C1. | 18 | Musk deer farm I, Chopta, Chamoli |
| C2. | 20 | Musk deer farm II, Chopta, Chamoli |
| C3. | 21 | Musk deer farm III, Chopta, Chamoli |

| | | |
|---|---|---|
| ***A. falcata*** | | |
| C4. | 24 | Joshi math Chandi I, Chamoli |
| C5. | 64 | Van I, Chamoli |
| C6. | 21 | Chopta I, Chamoli |
| C7. | 68 | Van II, Chamoli |
| C8. | 72 | Van III, Chamoli |
| C9. | 26 | Joshi math Chandi II, Chamoli |
| C10. | 22 | Chopta II, Chamoli |
| **T. spathiflorus** | | |
| D1. | 27 | Chopta forest I, Chamoli |
| D2. | 32 | Musk deer farm I, Chopta, Chamoli |
| D3. | 34 | Musk deer farm II Chopta, Chamoli |
| D4. | 81 | Thamrikund I, Munsyari, Pithoragarh |
| D5. | 111 | Bahli, Rampur, Shimla (H.P.) |
| D6. | 30 | Chopta II forest, Chamoli |
| D7. | 83 | Thamrikund II, Munsyari, Pithoragarh |
| D8. | 85 | Thamrikund III, Munsyari, Pithoragarh |
| D9. | 88 | Thamrikund IV, Munsyari, Pithoragarh |
| D10. | 105 | Taklech, Rampur, Shimla (H.P.) |

**Source: National Bamboo Mission Project Report (2011)**

# Results

Study was carried out for forty genotypes of four hill bamboo species conserved at Hill bambusetum, Khirsu, Uttarakhand. All genotypes represent different ecozones of Garhwal as well as North- Western Himalayas (Table 1).

## Polymorphism, Resolving power and polymorphic information content of markers identified

The isolated DNA from leaf samples of all forty genotypes by CTAB method ranged purity between 1.75-2.00 which was good enough for carrying out RANDOM analysis. The concentration of DNA was estimated in range of 55.0 ng /µl to 1302.0 ng /µl.

Initially, forty two (42) random primers were tested; only six polymorphic primers viz. OPG12, OPN06, OPR07, OPR08, OPA04 and were retained to carry out further analysis. The total number of scorable bands was 248 as amplification products of six random decamer primers. Thus, the average number of bands amplified per primer was 41.17 in all four populations of hill bamboo species. Out of 248 amplified bands, 190 bands were polymorphic whereas 58 were monomorphic.The mean number of polymorphic bands generated per primer was estimated 31.67. The average polymorphism of primers estimated was 76.55% in the range of 65.26%-89.38%. The primers with the high Rp values were more informative as they were able to distinguish more number of per genotypes. The mean value of resolving power (Rp) was 12.97. The maximum Rp value (14.95) was scored for OPG12 whereas minimum (10.90) was recorded in OPA17.Polymorphic information content ranged from 0.27 (OPN06) to 0.35 (OPR07) with an average value of 0.32 per primer (Table 2).

Table 2
List of random primers and amplification details used for the study of genetic parametersof hill bamboo species

| Primer | Primer seq. (5’ – 3’) | TNB | NPB | NMB | P % | Rp | PIC |
|---|---|---|---|---|---|---|---|
| **OPG12** | **5'CAGCTCACGA3'** | 48.00 | 36.00 | 12.00 | 76.19 | 14.95 | 0.33 |
| **OPN06** | **5'GAGACGCACA3'** | 41.00 | 35.00 | 6.00 | 85.60 | 12.80 | 0.27 |
| **OPR07** | **5'ACTGGCCTGA3'** | 42.00 | 27.00 | 14.00 | 65.26 | 13.90 | 0.35 |
| **OPR08** | **5'CCCGTTGCCT3'** | 44.00 | 32.00 | 12.00 | 71.31 | 13.80 | 0.28 |
| **OPA04** | **5'AATCGGGCTG3'** | 36.00 | 27.00 | 9.00 | 71.58 | 11.45 | 0.33 |
| **OPA17** | **5'GACCGCTTGT3'** | 37.00 | 33.00 | 5.00 | 89.38 | 10.90 | 0.34 |
| **Total** | | **248.00** | **190.00** | **58.00** | **-** | **-** | **-** |
| **Mean** | | **41.17** | **31.67** | **9.67** | **76.55** | **12.97** | **0.32** |

***TNB= Total number of bands; NPB = Number of polymorphic bands; NMB = Number of monomorphic bands; P% = Polymorphism percentage; Rp = Resolving Power; PIC= Polymorphic information content.**

The primer screening step in 42 decamer primers revealed GC content ranging from 60 to 70% for all the species. Only 20, 12, 25 and 28 primers exhibited good amplification for the species *A. falcata, T. falconeri, S. anceps* and *T. spathiflorus* respectively and continued for further analysis. The tested random primers allowed the discovery of high level of polymorphism. Average polymorphism values (%) were 73.50, 79.33, 83.11 and 85.11 for *A. falcata, T. falconeri, S. anceps* and *T. spathiflorus* respectively which was in contrast to the findings of Bhattacharya et al., (2006) who reported no polymorphism within species of B. tulda. Six polymorphic primers (OPG12, OPN06, OPR07, OPR08, OPA04 and OPA17) produced distinct, reproducible and polymorphic bands common among all four species. In the the present study, the phylogenetic relationships were estimated using more than adequate number of loci i.e. 42. In an analogous study, Nayak et al (2003) reported generation of 137 fragments from 12 bamboo

species using 10 random primers which were 100% polymorphic. The range of PIC value of primers were 0.20 to 0.39 (*A. falcata*), 0.16 to 0.41 (*T. falconeri*), 0.22 to 0.45 (*S. anceps*) and 0.21 to 0.44 (*T. spathiflorus*). Primer-3, OPG 02 and OPR-07 primers were most efficient primer with highest PIC (0.41, 0.44 and 0.45 respectively) and concluded that these primers were much informative to distinguish between genotypes. Resolving power (Rp) values of selected primers were quite higher with mean values of 11.95, 11.02. 12.18 and 12.78 for the species *A. falcata*, *T. falconeri*, *S. anceps* and *T. spathiflorus* respectively which in turn explain the efficacy of selected primer for genetic diversity.

# Genetic Diversity

The highly polymorphic six arbitrary decamers were selected for further PCR amplification. A total 248 DNA fragments were resolved, out of which 190 bands were highly polymorphic. The number of the polymorphic bands (np) generated by each used random primers ranged from 36 (OPG-12) to 27 (OPR-07 & OPA-04) (Fig. 1). The percent of polymorphism (P%) varied from 65.26% (OPR- 07) to 89.38% (OPA-17) with average 76.55% polymorphism. The various diversity parameters were estimated to assess the genetic diversity revealed in genotypes of all four bamboo species. The observed number of alleles (na) estimated was2.0. The highest value estimated for the species *T. falconeri* (1.7162) and minimum in the genotypes of *T. spathiflorus* (1.473); however the effective number of alleles (ne) [Kimura and Crow (1964)] was found to be 1.6937 (*A. falcata*, 1.353; *T. falconeri*, 1.5151; *S. anceps*, 1.3945 and *T. spathiflorus*, 1.3084). The Nei's (1973) gene diversity (h) were estimated among genotypes as 0.2041, 0.2901, 0.2173 and 0.1798 in*A. falcata*, *T. falconeri*, *S. anceps* and *T. spathiflorus* respectively with overall value 0.3915. The overall value of Shannon's Information index (I) [Lewontin (1972)] and Gene Diversity in Subdivided Populations (Nm) were estimated as 0.5726 and 0.6605 respectively (Table 3).

Table 3
The genetic diversity estimates of hill bamboo species

| Sl. No. | Species | na | ne | h | I | Gst | Nm |
|---|---|---|---|---|---|---|---|
| 1 | *A. falcata* | 1.527 | 1.353 | 0.2041 | 0.3011 | 0.4308 | 0.6605 |
| 2 | *T. falconeri* | 1.7162 | 1.5151 | 0.2901 | 0.4231 | | |
| 3 | *S. anceps* | 1.527 | 1.3945 | 0.2173 | 0.315 | | |
| 4 | *T. spathiflorus* | 1.473 | 1.3084 | 0.1798 | 0.2664 | | |
| 5 | *Overall* | 2 | 1.6937 | 0.3915 | 0.5726 | | |

***na= observed number of alleles, ne = Effective number of alleles [Kimura and Crow (1964)], h = Nei's (1973) gene diversity, I = Shannon's Information index [Lewontin (1972)], Nm (Gene Diversity in Subdivided Populations)=Estimates of gene flow from Gst or Gcs**

# Discussion

The RAPD technique described by Stange et al. (2004) provided the basis for developing a standardized protocol suitable for the experimental species. Analysis of genetic variation in populations of four montane bamboo species demonstrated high genetic diversity within species (63%). Elevated genetic diversity within populations has been reported in Thamnocalamus spathiflorus (Tiwari et al., 2019) using RAPD markers, Dendrocalamus membranaceus using ISSR markers (Yang et al., 2012), and M. baccifera (Jeeva et al., 2009). Ramanayake et al. (2007) also investigated diversity and relationships among nine bamboo species using RAPD markers. Long-lived, outcrossing, and self-incompatible plants typically exhibit higher genetic variation at the species level and lower differentiation among populations (Hamrigk and Godt, 1996; Zawko et al., 2001). Nybom (2004) similarly reported that most genetic variability is retained within populations of long-lived and outcrossing plant species, as demonstrated by RAPD and sequence-tagged microsatellite site (STMs) analyses. Species with broad geographic ranges tend to maintain higher genetic diversity than those with restricted distributions (Chen et al., 2014). The observed low genetic diversity among species (37%) suggests that hill bamboo species are confined to small, localized areas within their natural range. Genetic diversity within populations is influenced by factors such as mating system, population size, prolonged periods with low numbers of individuals, genetic drift, and gene flow (George et al., 2009). High genetic diversity within small populations may also occur if population size reduction has taken place recently, particularly within one or two generations (Zhao et al., 2012). In these cases, surviving individuals effectively represent samples from previously larger populations. The presence of significant genetic variation within the studied hill bamboo populations may indicate an eroding genetic base in the natural populations of these species. A previous study of *Thamnocalamus spathiflorus* natural populations, based on morphological and molecular (RAPD) markers, found no significant diversity within populations of *T. spathiflorus* (Bhattacharya et al., 2009). Tiwari et al. (2015) examined the taxonomic characteristics and genetic profile of the mountain bamboo species Thamnocalamus falconeri.

The value of the coefficient of genetic differentiation among populations (Gst) was 0.4308, a figure which is higher than the mean value (Gst 0.073) obtained for 121 species of woody plants studied using allozyme markers (Hamrick et al., 1992); Yang et al. (2012) likewise found a lower Gst (0.252) in their ISSR analyses of *D. membranaceus* and *M. baccifera (*Nilkanta et al., 2017).

# Molecular Variance (AMOVA)

The fact that the Gst value is low in the hill bamboos shows that there is a low degree of genetic differentiation among the populations. This is also supported by the analysis of molecular variance (AMOVA), since this analysis too shows only limited genetic differentiation. There is a negative correlation between genetic differentiation among populations and gene flow (Grant, 1991). The results are in agreement with the Nm value of 0.6605, which is much higher than the Nm value of 0.0101 given for M. baccifera by Nilkanta et al., 2017. Gene flow between populations can be achieved through both pollen and seed dispersal, as these two factors have a significant effect on plant evolution (Hamrick, 1987). The low estimates of gene flow (Nm) in the hill bamboos indicate that, because of the limited

possibility of sexual reproduction, these four bamboo species mainly spread by means of vegetative propagation via rhizomes.

Geographic isolation is a major factor in causing genetic differentiation among populations since it limits gene flow both by means of pollen and seeds as well as through human activity (Pfeifer and Jetschke, 2006). The low value for gene flow found in this study (Nm 0.6605) also confirms the conclusion that genetic differentiation among the hill bamboo populations is due to geographic isolation.

## Conclusion

The genetic resources of montane bamboos have been extensively exploited, resulting in resource reduction, habitat destruction, and fragmentation. The significant decline in natural bamboo populations, prompted by increasing demand for their multiple uses, highlights the vital need for effective conservation measures. Although the studied hill bamboo species are closely related and exhibit considerable intra-specific diversity, genetic variation among species remains low. Conservation programs has to prioritize the preservation of individuals within existing populations. Scientific harvesting and sustainable management practices are recommended to ensure resource application without further population decline, hence maintaining the genetic base and structure of bamboo species. In situ conservation of remaining habitats should be complemented by ex situ strategies, including seed and vegetative propagation through culm cuttings, division, and tissue culture. Increasing awareness among local communities about the genetic status of bamboo populations and integrating them in the development of protection policies will improve the effectiveness of conservation initiatives.

## Declarations

# Consent to Publish declaration

not applicable.

**Conflict of interest:**

No financial or personal interests could influence the authors' work.

# Ethics and Consent to Participate declarations

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# Author Contribution

CT designed the study, conducted fieldwork, and prepared the draft manuscript. KS and DG compiled, analysed the data and contributed to the manuscript draft for publication. MB supervised the study and contributed to the manuscript draft. SK MB reviewed the entire manuscript draft.

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# Data availability statement:

not applicable. This manuscript does not report data generation or analysis.

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# Figures

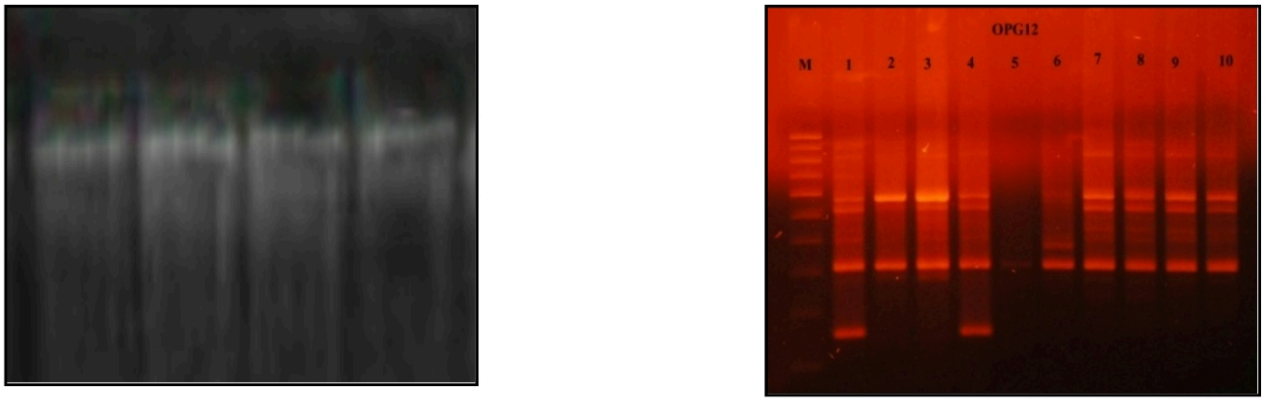



**Figure 1**

**Genomic DNA and DNA Profile**

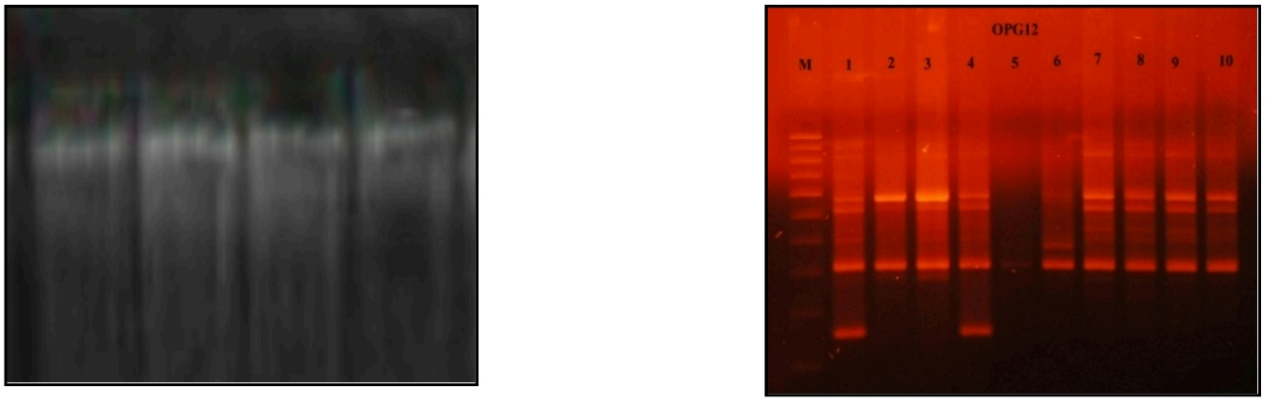



**Figure 1**

## Genomic DNA and DNA Profile

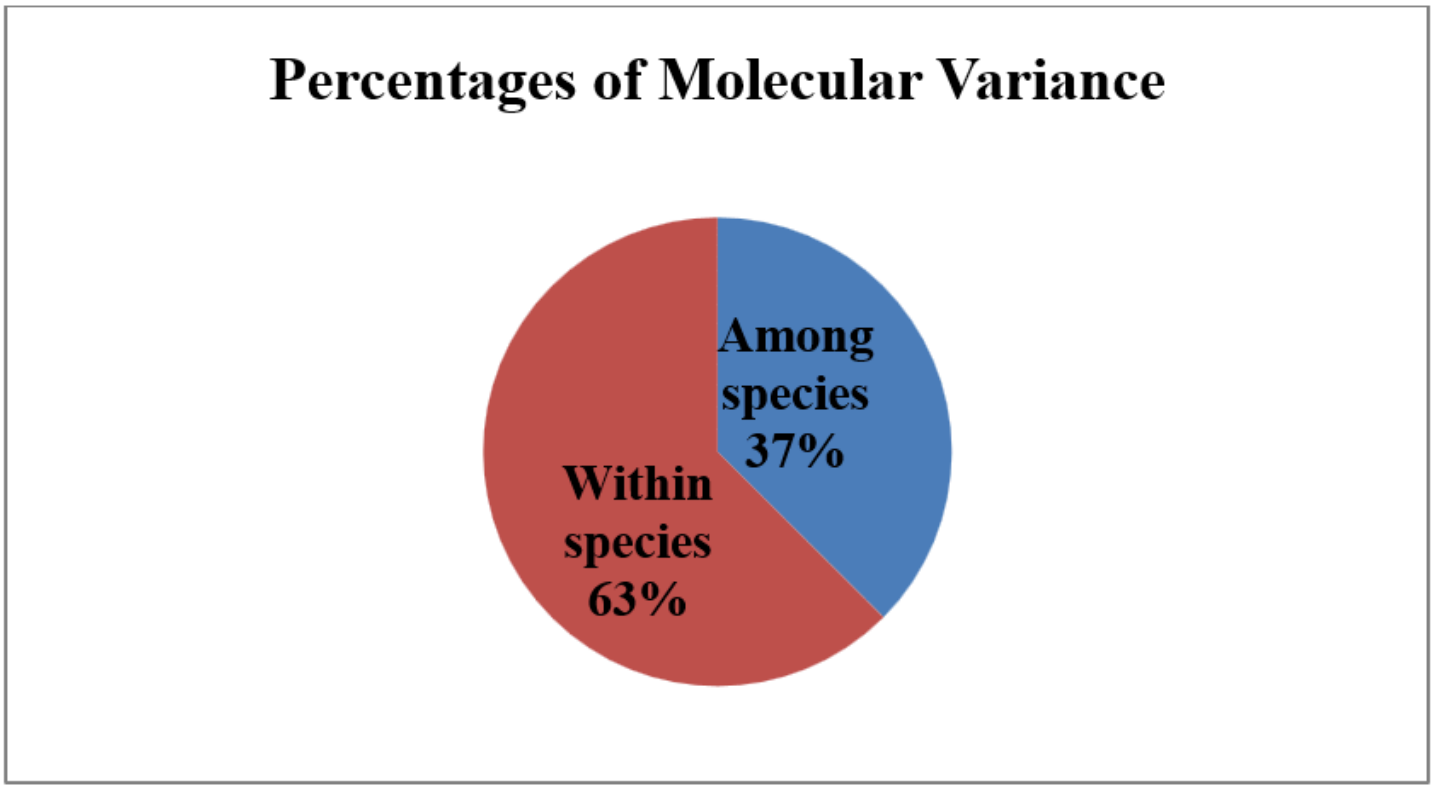



Figure 2

**Molecular variance analyses of four hill bamboo species**

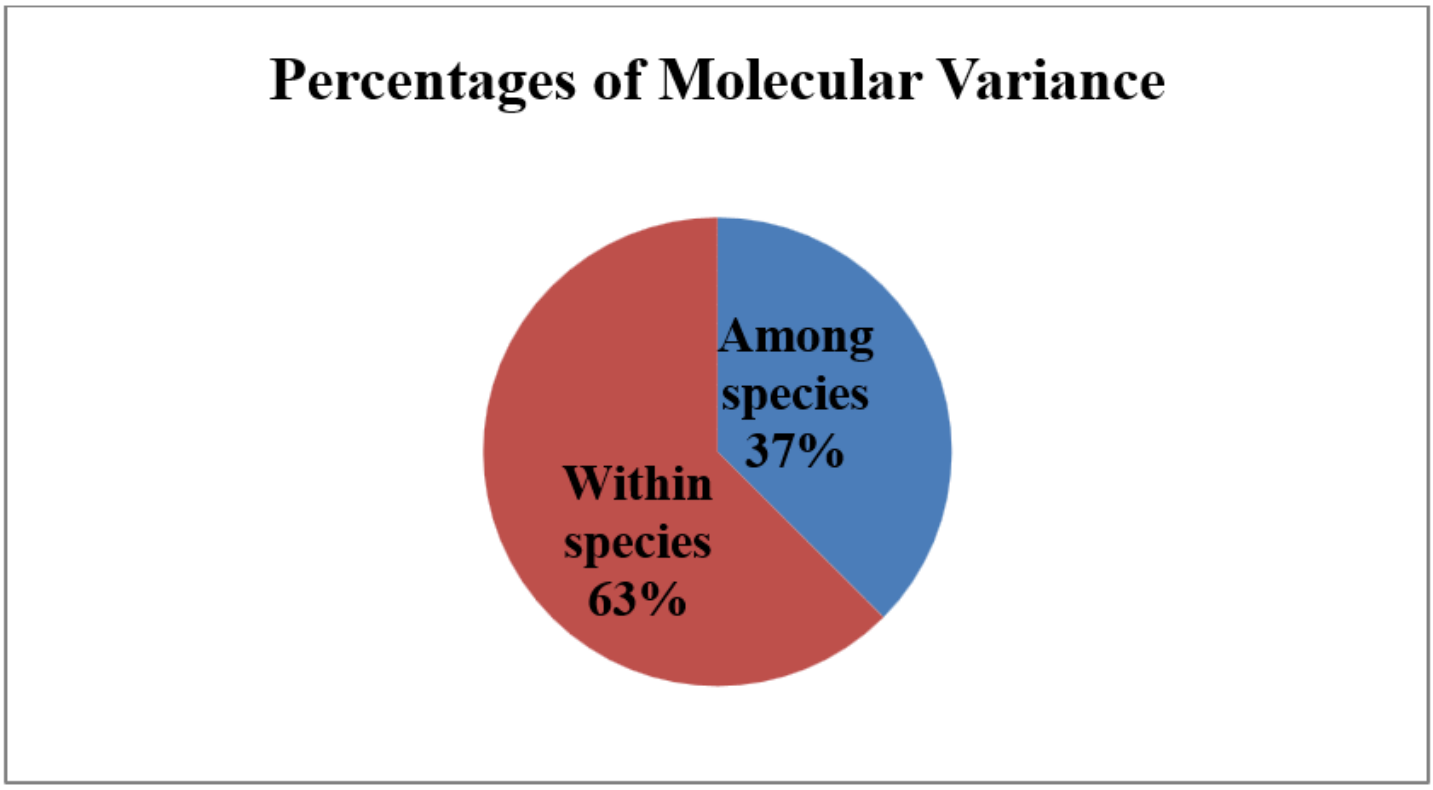



**Figure 2**

**Molecular variance analyses of four hill bamboo species**

## Supplementary Files

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- photoplateRAPD.docx
- photoplateRAPD.docx