The bamboos, a group of tall arborescent grasses, have age-old connections with the material needs of rural people. Because of their multiple uses, bamboos continue to play an important role in the economy and life style of many Asian countries (Das et al., 2008). The bamboo subfamily (*Bambusoideae*, of the family *Poaceae*) comprises approximately 70 genera and 1,200 species worldwide (Wang and Shen, 1987). Most of the recent classification systems have placed 67 genera of woody bamboos in nine subtribes, based mostly on floral characters (Dransfield and Widjaja, 1995; Li, 1997). India harbors about 130 species representing 18 genera of Bambusoideae and is, therefore, considered one of the hotspots of bamboo genetic resources (Kumar, 2004).

Bamboo is an important plant species which has been and is still playing important role in the socio–economy of the people especially in rural areas and constitutes in integral part of their culture. Bamboo is called as “Bio – Steel”, “Green Gold”, “Poor man’s Timber”. It is often associated with prosperity, peace, good fortune, success and protection from evil. Despite its importance the plant is not fully explored due constraints of its flowering behavior, characterization and thus proper identification. Still a number of efforts have been made by the scientific community for the same.

Bamboos are vital to many Asian economies, having important uses ranging from domestic items to rural housing and raw materials for industry (Dransfield and Widjaja, 1995). However, overexploitation and genetic erosion of bamboo species have made it necessary to
collect germplasm for conservation purposes. In line with the collection of germplasm, greater attention is needed in the classification and identification of bamboos (Rao and Rao, 1995).

2.1 SYSTEMATIC POSITION OF BAMBOOS

Traditionally, the members of the group share some common features that include rhizomatous habit, hollow segmented culms, petiolar blade with tessellate venation, flowers with three or more lodicules, usually with six stamens, and fruit possess small embryo and linear hilum (Soderstrom, 1981). Few synapomorphic features which are unique for Bambusoideae were reported by GPWG (2001). Leaf blade is mainly constituted of mesophyll tissue with asymmetrically invaginated arm cells, while pseudo-petiole structures are secondary gain for the sub-family. It is broadly divided into two tribes that are Bambuseae/woody bamboos and Olyreae/herbaceous bamboos depending on the presence (Bambuseae) or absence (Olyreae) of the abaxial ligule (GPWG, 2001; Zhang and Clark, 2000).

Bamboo can be classified as woody, herbaceous or liana according to ITS growth characteristics, and grows predominantly in tropical and subtropical regions although a few species grow in temperate and frigid zones (Chen et al., 2003). Many different bamboo cultivars with diverse phenotypes have been produced during ITS long cultivation history (Dransfield and Widjaja, 1995; Fu, 2001).

Most of the recent classification systems (Dransfield and Widjaja, 1995; Li, 1997; Soderstrom and Ellis, 1987) placed 67 genera of woody bamboos in nine sub-tribes. These classification systems were largely dependent on various floral characters such as type of inflorescence or ovary appendages.
I. SUBTRIBE ARTHROSTYLIDIINAE:

II. SUBTRIBE ARUNDINARIINAE:

III. SUBTRIBE BAMBUSINAE:

IV. SUBTRIBE CHUSQUEINAE:
37. Chusquea, 38. Nerolepis

V. SUBTRIBE GUADUINAE:

VI. SUBTRIBE MELOCANNINAE:
VII. SUBTRIBE NASTINAE:

VIII. SUBTRIBE RACEMOBAMBOSINAE:
60. Racemobambos (Neomicrocalamus)

IX. SUBTRIBE SHIBATAEINAE:

The bamboo subfamily, Bambusoideae, is one of the five subfamilies of the grasses (Poaceae) (Soderstrom and Ellis, 1986). The Bambusoideae are currently divided into the tribes Bambuseae and Olyreae; woody bamboos (Bambuseae) consist of approx. 77 genera and 1030 species worldwide (Dransfield and Widjaja, 1995). The supertribe Bambusodae is further subdivided into nine subtribes, one of which is Bambusinae, consisting of ten to 13 genera, found mostly in tropical Asia (Dransfield and Widjaja, 1995).

2.2 PROBLEMS IN BAMBOO CLASSIFICATION

Bamboos flower once in a lifetime and then die: the vegetative phase lasts from 1–120 years depending on the species. Because of this peculiar and uncertain nature of flowering, vegetative characteristics such as rhizomes, culms, culm sheaths, ligules, branching, and leaves have been traditionally used to classify and establish phylogenetic relationships among the bamboo genera (Ohrnberger and Goerrings, 1986; Isagi et al., 2004; Sharma et al., 2008).

It has been difficult to relate these characteristics to floral distinctions, which is one cause of the controversy in the classification of Bambusa and many other bamboo genera. Sharma et
(2008) also reported that the taxonomic demarcation of woody bamboos at lower ranks, such as genera and species, is not well resolved to date. Quite often, morphological characters are influenced by environment and are complex, limited in number, and state-specific in their expression, making an objective analysis difficult (Wu, 1962). This has led to the misclassification of bamboo genera and species.

Basic knowledge of the biology and genetics of bamboo is severely lacking. This is a direct result of the unusual life cycle of bamboo. Among bamboo species, the vegetative growth phase varies from 1 year to as long as 120 years, and some species have never been known to flower (Janzen, 1976). Identification of sterile plants is therefore problematic as taxonomic studies of bamboos have traditionally depended heavily on inflorescence and floral morphology because:

(1) Vegetative characters are often environmentally influenced, which makes them less constant for systematic purposes (Wu, 1962);

(2) Characters that delimit species may be more subtle and not available for study; and

(3) Bamboo clones found in Asia are selected for economic value and are widely distributed without proper identification at the species level.

2.3 RELEVANCE OF MOLECULAR SYSTEMATICS

Two major objectives of any taxonomic study are (a) systematic grouping of the taxa of interest through generation of robust, natural classification system based on stable characters that reflect their true evolutionary history and (b) development of reliable identification key(s) for easy taxon determination.

Most of the classifications proposed to date for bamboo are primarily dependent on various morphological features and one of the most immediate needs is to test how natural all
these systems are. Stapleton (1997) has summarized few important limitations associated with the traditional morphological classifications:

(1) Morphology-based classifications are often superficial as similarities have frequently gained priorities over dissimilarities.

(2) Reproductive characters have often earned priority with an assumption of having higher evolutionary significance than the vegetative characters. The importance of many vegetative features such as rhizome or branch patterns was understood later and thus many of the early herbarium specimens were incomplete.

(3) In many cases artificiality was enhanced as characters were frequently considered in isolation rather than considered in groups. It is undeniable that vegetative features are quite essential for field identification of the woody members as flowering cycles are often erratic, which severely restricts the opportunity to study fresh reproductive materials. Even if the dried, herbarium samples are available, quite often these lack enough morphological resolution and thus create confusion in the real field condition.

Hence, the identification keys are mostly dependent on various vegetative features that need further refinement and reinvestigation. In particular, the taxonomic demarcation of woody bamboos at lower ranks, such as genera and species, are not well resolved to date. There are several species which are known only vegetatively, new species are constantly been described (Filgueiras and Londono, 2006; Triplett, et al., 2006; Clark et al., 2007) and several undescribed taxa are known to occur in the wild habitat of South and Central Americas.

Morphological classification is mostly dependent on vegetative characteristics that are easily influenced by environmental factors (Ramanayake et al., 2007). The alternative is the use of molecular systematics based on methods of identifying DNA polymorphisms between
individuals or species to overcome these problems. DNA markers therefore provide an alternative approach for studying bamboo genetic diversity, genetic distance analysis, and species identification. Although molecular data have been used to show relationships between the temperate, tropical New World and Old World woody bamboos, further resolution within the subfamily *Bambusoideae* is needed (Clark, 1997; Kobayashi, 1997).

It is clear that molecular data sets can provide useful information for addressing various aspects of plant taxonomy. The major challenge associated with any molecular method is however, to determine the appropriate taxonomic level at which it is most informative and to correlate it with morphologically definable taxonomic groupings.

However, application of molecular techniques for the study of genetic diversity in bamboo has been limited. Studies include the use of restriction fragment length polymorphisms (RFLP) in *Phyllostachys* (Friar and Kochert, 1991, 1994), isozyme analysis of a limited selection of bamboos from five genera (Heng et al., 1996), chloroplast DNA phylogeny of Asian bamboos (Watanabe et al., 1994) and world bamboos (Kobayashi, 1997), and the use of chloroplast *rpl16* intron sequences in determining phylogenetic relationships within the genus *Chusquea* (Kelchner and Clark, 1997).

2.4 GENOME SIZE AND DNA CONTENT IN BAMBOOS

Bamboos are polyploids; temperate bamboos have 48 chromosomes, tropical ones have 72 chromosomes, so they are tetra- and hexaploids respectively, assuming a basic chromosome number of *x* = 12 in grasses. The chromosomes of tropical bamboos are very small and the karyotypes of temperate bamboos are very complicated (Kondo, 1964). Also given the lack of breeding systems, it seems almost impossible to do any serious study on the genome of bamboo.
But in plants, and more specifically in grasses, large parts of the genome are collinear, that is, genes are arranged in similar way on chromosomes, and there is quite a large degree of genome conservation. If this were true, one might exploit the knowledge generated in e.g. rice, to study the genetics of bamboo, since rice and bamboo are very closely related within the grass family. But as a first step it is necessary to estimate the complexity of the genome. To this aim flow cytometry was used, which yielded some very interesting results (Gielis et al., 1997c).

There are two main groups in bamboo, the temperate bamboos with DNA contents of 4-5.5 pg/2C, and the tropical bamboos with about 2.5-3.2 pg/2C. If calculated per 12 chromosomes (one «complement ») the DNA content of tropical bamboos is very similar to that of the haploid genome of rice (x = n = 12). Genome size has little connection to size of plants, as *Phyllostachys* has the lowest DNA content in temperate bamboos, and the herbaceous bamboo *Lithachne humilis* (3.69 pg/2C) has a value intermediate between tropical and temperate bamboos. Endoreplication of DNA in leaves of flowering *Fargesia murieliae* was observed. The results also suggest that polyploidy has been one of the major driving forces in the evolution of woody bamboos.

According to previous studies, the DNA contents of bamboo were 2.45—5.3 pg DNA/2C, and 4.17—5.3 pg for the temperate bamboo (*Phyllostachys*) (Gielis et al.,1997). This implies that bamboo genomes are relatively large within the grass family. Bamboo is a special node in plant genomics, particularly in poaceous genomics. Although massive genomic sequences have been determined in the *Poaceae* family, some of its key nodes are still blank, such as bamboo. Most Poaceous genes are identifiable through large-scale sequencing. However, there remain many gaps in knowledge of intragenic variation across different species of the family, in which the bamboos represent an important branch (Paterson et al., 2005).
In Moso bamboo (*Phyllostachys pubescens*) bamboo genome size was estimated to be about 2034 Mb by flow cytometry (FCM), using maize (cv. B73) and rice (cv. Nipponbare) as internal references (Gui et al., 2007). They found that the size of the moso bamboo genome was similar to that of maize but significantly larger than that of rice. To determine whether the bamboo genome had a high proportion of repeat elements, similar to that of the maize genome, approximately 1000 genome survey sequences (GSS) were generated. Sequence analysis showed that the proportion of repeat elements was 23.3% for the bamboo genome, which is significantly lower than that of the maize genome (65.7%). The bamboo repeat elements were mainly Gypsy/DIRS1 and Ty1/Copia LTR retrotransposons (14.7%), with a few DNA transposons. However, more genomic sequences are needed to confirm the above results due to several factors, such as the limitation of our GSS data. This study was the first to investigate sequence composition of the bamboo genome with valuable clues for future genome research of moso and other bamboos.

2.5 BIOCHEMICAL BASED MARKERS

Isozyme markers have been widely used extensively to identify and discriminate cultivars in many agricultural and horticultural crops, in forestry or identifying clones. Application of isozymes for identification of bamboo clones has been done efficiently. Chu et al. (1972) identified *Dendrocalamus latiflorus* clones in Taiwan employing peroxidase enzyme. A total of eight bamboo categories were obtained among 85 clones.

Identification of bamboo cultivars based on isozymes pattern was carried out by Samart (1993). Polyacrylamide gel electrophoretic technique using leaf extract of nineteen cultivars of 7 genera (*Bambusa, Dendrocalamus, Semiarundinaria, Cephalostachyum, Thysostachys, Arundinaria and Guadua*) and 13 cultivars of 6 genera (*Bambusa, Dendrocalamus,*)
Cephalostachyum, Thyrsostachys, Phyllostachys and Chimonobambusa) was utilized to study 3 enzyme systems namely acid phosphatase, esterase and peroxidase. The isozyme patterns of all enzyme systems showed differences among genera and the species within the genera Bambusa and Dendrocalamus. It was also found that season and growing location affected isozyme patterns.

Hsieh et al. (2006), performed a study on molecular cloning and functional identification of invertase isozymes from green bamboo Bambusa oldhamii. Three Bo beta fruct cDNAs encoding acid invertases were cloned from shoots of the green bamboo Bambusa oldhamii. On the basis of the amino acid sequences of their products and phylogenetic analyses, Bo beta fruct1 and Bo beta fruct2 were determined to encode cell wall invertases, whereas Bo beta fruct3 encodes a vacuolar invertase.

2.6 MOLECULAR MARKERS

Considering the difficulties in identification of genotypes in bamboos, the use of molecular markers, may turn out to be very useful. As a tool, molecular markers will save lots of time, although careful consideration must be made when and where to apply which method.

Though molecular data sets can provide useful information on various aspects of taxonomy, there is limited information available on genetic diversity or population structure of bamboos. In the last ten years we have already witnessed several generations of molecular markers, which become increasingly precise, but also require a new approach every time. Starting with isozymes and proteins, in the second half of the eighties restriction digestion and visualization of DNA fragments obtained by restriction digestion (RFLP) were used widely, also in bamboo. The big boom of molecular markers came with PCR-technology, with Random Amplified Polymorphic DNA and related techniques (Gielis et al., 1997). Molecular techniques
based on polymerase chain reaction (PCR), such as random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), simple sequence repeats (SSR), and amplified fragment length polymorphism (AFLP), are being used for phylogenetic studies in temperate bamboo (Friar and Kochert, 1994; Kobayashi, 1997; Barkley et al., 2005).

More recently DNA sequences have been used to infer phylogenetic relationships in the bamboos, but only limited sequence variation has been found in the genes studied (Zhang, 1996; Hodkinson et al., 2000; Guo et al., 2001). Das et al. (2005) developed two species-specific sequence characterized amplified regions (SCARs) for differentiating Bambusa balcooa and B. tulda species. The use of SSR markers is limited by their lack of availability in bamboo; nevertheless, they have been successfully applied to Phyllostachys (Lai and Hsiao, 1997) and Bambusa (Nayak and Rout, 2005). Unlike the abundance of genetic information now available on various dicotyledonous model plant species, for example, Arabidopsis thaliana (Arabidopsis Genome Initiative 2000), and the monocotyledonous model plant species Oryza sativa (International Rice Genome Sequencing Project, IRGSP), there is relatively little information available on the genome of bamboo. The plant databases currently available for rice and other monocotyledonous plants are not suitable for studying bamboo. Consequently, it is necessary to develop biotechnological tools and a database specific for bamboo in order to further our understanding of the genome of this plant.

These molecular studies thus bring to light the problematic areas in the classification of certain bamboo species at the generic level. These problems may be due to inconsistencies in morphological descriptions of the inflorescence that were used in their generic assignments.
Among the use of molecular marker, the most simple, fast and easy to perform assay is the use of RAPD markers to determine the existing genetic diversity and variation among and within the population (Kumari and Pande, 2010).

Friar and Kochert (1991, 1994) used nuclear Restriction Fragment Length Polymorphism (RFLP) to study the variation and evolution in 20 species and 61 accessions of Phyllostachys, a group of temperate bamboos. Kelchner and Clark (1997) used chloroplast gene rpl16 to study the genetic diversity of Chusquea and Bambusoidea.

Genetic Variation and Relationships within the four genera of bamboos (Bambusa, Dendrocalamus, Gigantochloa and Thyrsostachys), Subtribe Bambusinae were studied using Amplified Fragment Length Polymorphism (AFLP) by Loh et al. (2000). Eight primer combinations was carried out on 15 species of bamboo to distinguish different species by their unique banding patterns. Unique AFLPs were detected in 13 of the 15 species examined. The six Bambusa species separated into two clusters, six Gigantochloa species formed a discrete cluster diverging from one of the Bambusa clusters, while Thyrsostachys was less similar to the Bambusa clusters. The similarity index between B. lako and G. atroviolacea was the highest, suggesting that B. lako is more appropriately included within the genus Gigantochloa rather than the genus Bambusa. The two Dendrocalamus species examined were very different with D. brandisii clustering within one of the Bambusa clusters and D. giganteus appearing as a very distant species. These results support the contention that critical study of the genus Dendrocalamus is required.

Loh et al. (2000) used AFLP markers to study the relationship between four genera of woody bamboos. AFLP has proved to be useful in diverse aspects of bamboo systematics, population structure, and diversity studies (Loh et al., 2000). AFLP (amplified fragment length
polymorphism) molecular markers have also been used to compare species of *Phyllostachys* (Hodkinson *et al.*, 2000) and species of *Bambusa* and closely related genera from Singapore Botanic Gardens (Loh *et al.*, 2000). RFLP and AFLP markers may provide a larger number of polymorphic markers than RAPD but are also often more expensive and technically demanding.

Nayak *et al.* (2003) evaluated the genetic variability in 12 species of bamboo using RAPD markers. A total of 137 distinct polymorphic DNA fragments (bands), ranging from 0.4–3.3 kb using 10 selected primers, revealed a wide range of variability among the species. Cluster analysis clearly showed two major clusters, further divided into three minor clusters. *Bambusa vulgaris* and *Bambusa vulgaris* var. *striata* were the most closely related and formed the first minor cluster along with *Bambusa ventricosa*. The variety of *Bambusa multiplex* var. *Silver stripe* and *Bambusa multiplex* were very closely related and there was no variation with *Bambusa ventricosa*. Another minor cluster was obtained between *Bambusa arundinacea, Cephalostachyum pergracil* and *Bambusa balcooa*.

Thirty clumps of six populations of *Bambusa pervariabilis* were analyzed by RAPD markers to determine the genetic variations among and within the populations (Xing *et al.*, 2003). A total of 173 loci including 85 polymorphic ones were amplified using 28 random primers, with an average of 6.18 fragments for each primer. The length of fragments was between 200 bp and 2000 bp. Six populations had an average Nei's gene diversity of 0.2114, and Shannon's genetic diversity of 0.3277, coefficient of gene differentiation (Gst) of 0.1853, indicating that there was some differentiation among the populations. The average genetic distance among populations was 0.0350, indicating that there was close relative relations among populations. The six populations were clustered to three categories by cluster analysis (UPGMA) based on Nei's unbiased genetic distance.
Thirty different bamboo germplasm collected from different parts of North East India were used for random polymorphic DNA (RAPD) analysis to determine genetic diversity and establish phylogenetic relationship (Deka and Sharma, 2003). Out of twenty random primers used to screen the germplasm, 15 primers generated 1361 reproducible band out of which 1093 were found to be polymorphic. The level of polymorphism was found to be 80.30%. The dendogram, based on Jacard’s index, revealed three major groups. The groupings indicated uniformity in their geographical origin that is, the species collected from a particular region were found to cluster in the same groups. They concluded that large genetic variation existed among the bamboo species of North East India.

Polymorphic EST–SSR markers derived from major cereal crops have recently been used to assess phylogenetic and genetic diversity (Barkley et al., 2005; Sharma et al., 2008). Das et al. (2005) succeeded in development of species specific SCAR markers which were able to identify *Bambusa balcooa* and *B. tulda* using the technique of cloning and sequencing of RAPD products to allow for their proper identification, in order to avoid unintentional adulteration that affects the quality and quantity of paper pulp production. Two putative, species-specific RAPD markers, Bb836 for *B. balcooa* and Bt609 for *B. Tulda* were generated using a PCR-based RAPD technique. Sequence-characterized amplified region (SCAR) markers were developed from Bb836 and Bt609 sequences, using 20-mer oligonucleotide primers designed from both the flanking ends of the respective RAPD primers. The species-specific SCAR fragments were named as ‘Balco836’ for *B. balcooa* and ‘Tuldo609’ for *B. tulda*. The species-specific ‘Balco836’ was amplified from the genomic DNA of 80 individuals of 16 populations of *B. balcooa* studied. Similarly, the presence of ‘Tuldo609’ was noted in all the 80 individuals representing 16 populations of *B. tulda* assessed. They concluded that the two molecular markers
were potentially useful for regulatory agencies to establish sovereign rights of the germplasms of *B. balcooa* and *B. tulda*.

The elite clones of *Dendrocalamus strictus* and *Bambusa bambos* were analyzed using RAPD markers. Out of 80 random primers, 42 in *D. strictus* and 32 in *B. bambos* produced polymorphic banding patterns. A genetic similarity range of 61.40% to 84.23% in *D. strictus* while 51.58% to 93.11% in *B. bambos* was observed and cluster analysis grouped eleven clones of each species into three major groups (Biradar *et al.*, 2005).

Lin *et al.* (2006) identified bamboo genes expressed differently in an albino mutant and wild type *B. edulis* using ESTs (Expressed Sequence Tags). Lin *et al.* (2006) showed that use of ESTs (Expressed Sequence Tags) enabled the identification of genes involved in causing an albino mutant character in *B. edulis* shoots raised in tissue cultures. Similar studies may enable detection of polymorphic genes in forms of bamboo species, which are not detected by RAPD. Bhattacharya *et al.* (2006) reported that in *B. tulda* populations, some clumps exhibit the bulbous internode character as well as differences in culm colour. However, he reported that these forms did not show polymorphisms with RAPD markers.

Das *et al.* (2007) studied phylogenetic relationships among 15 bamboo species using 32 key morphological descriptors and 120 polymorphic loci of the genomic DNA generated using RAPD technique. Phylogenetic relationships as revealed from the dendrogram and principal component analysis was in concurrence with the reliable, widely referred system of bamboo classification, while, the cluster pattern generated from the similarity matrix derived from key morphological character analysis was discriminatory. They concluded that the molecular evidences need to be supplemented by morphological data to validate the phylogenetic relationships among taxa.
The genetic diversity in *Neosinocalamus affini* and *Bambusa rigida* were studied by RAPD-PCR in different areas of Sichuan Province, China (Xiaoyi, 2007). Fifty of amplified locus were obtained. Of them, 42 of amplified locus had polymorphism. The 7.1 number of bands were amplified by one primer with 6 of exhibiting polymorphism. The genetic distance between the same type of bamboo from the different regions ranged from 0.1278 to 0.6539 indicating that the genetic diversity was rich for the same type of bamboo in the different areas. However, the genetic distance between *Neosinocalamus affini* and *Bambusa rigida* was far from 0.4463 to 0.9163.

Genetic diversity and relationships between nine species of bamboos in Sri Lanka was studied using RAPD markers (Ramanayake, et al., 2007). They were *Dendrocalamus giganteus* Wall ex Munro, *D. asper* Becker ex K. Heyn, *D. longispathus* (Kurz) Kurz, *Bambusa vulgaris* `Striata', *B. ventricosa*, *B. bambos* (L.) A. Voss, *B. atra* Lindley, *Gigantochloa atroviolacea* Wdjaja and *Arundinaria hindsii*. The lowest mean genetic distance of 0.143 was obtained between *B. vulgaris* and *B. ventricosa*. The genetic distances separating *D. longispathus* from *D. giganteus* and *D. asper* were both greater than that between the latter two species, which had a value of 0.313. Although the genetic distances between *Bambusa vulgaris*, *B. ventricosa* and *B. bambos* were relatively small, *B. atra* separated from them at greater distances. The smaller genetic distances between *G. atroviolacea* and *B. vulgaris*, *B. ventricosa* and *B. bambos* indicated that *G. atroviolacea* had a closer affinity to these three *Bambusa* species than *B. atra*. *A. hindsii* with the greatest genetic distances from all other species in the study was not related to any of them.

Eevera et al. (2008) used RAPD for analysis of genetic variation and differentiate among 26 bamboo species. Screening was done with 50 random primers and consistent results
were obtained with about 10 primers. *Dendrocalamus* species stood out among other bamboo species.

The use of ISSR markers in bamboo is limited to cultivars of *Phyllostachys pubescens* (Lin et al., 2009). The article reports the phylogenetic relationships and genetic diversity assessment in 22 bamboo taxa (20 species) as revealed through ISSR and EST-based random markers.

Identification of the hybrid bamboo F1 by SSR markers was done by JiangJie et al. (2009). Three hybrid bamboo candidates (*Pleioblastus simonii* × *Phyllostachys violascens*, *Sasa tokugawana* × *S. borealis*, and *Sinobambusa tootsik* × *P. distichus*) were identified using 10 SSR markers developed from genome survey sequences of *P. pubescens* in GenBank which were used to amplify the cross species. DNA fingerprinting of the 3 hybrid bamboo candidates showed a complementary pattern from female and male parents at the PBM014, PBM025 and (or) PBM018 locus. The sequencing and alignment of fragment DNA bands at the mentioned-above loci confirmed the homology between the hybrid bamboo candidates and their parents, indicating the validity of the hybrid bamboo results. This is the first report for the identification of bamboo hybrid using DNA markers.

Another work was conducted on 25 microsatellite loci for Fang’s cane bamboo *Bashania fangiana* by Zhan et al. (2009) When tested 31 with 21 samples from Labaha Nature Reserve, China, these loci exhibited a mean of 10 alleles 32 per locus, a mean expected heterozygosity of 0.789 and a mean observed heterozygosity of 33 0.708. All loci except two were in Hardy-Weinberg equilibrium and no consistent evidence for 34 linkage disequilibrium was detected between any pair of loci.
Ten polymorphic microsatellite markers were isolated from the dwarf bamboo species *Sasa cernua* and *Sasa kurilensis* in northern Japan by Kitamura *et al.* (2009). The applicability of these markers was confirmed by genotyping of open-pollinated seeds and leaf samples from natural populations. Genotypes of seeds from each culm shared at least one allele from the maternal parent without contradiction. All 10 loci were polymorphic in *S. cernua* with 2–15 alleles (average $H_E = 0.532$). Eight loci were polymorphic in *S. kurilensis* with 2–10 alleles (average $H_E = 0.532$).

Transferability of rice SSR markers to bamboo was done by Chen *et al.* (2010). They selected 120 rice SSR markers that are evenly distributed on rice chromosomes and assessed these for their transferability to 21 different bamboo species. A total of 4847 bands of 2196 alleles were obtained from 82 SSR markers that were able to amplify products in the bamboo genome; the transferability was 68.3%. Seven markers specifically amplified individual bamboo species and are consequently valuable markers for species identification. SSR markers located on rice chromosome 7 and 1 showed the highest and lowest transferability, respectively to the bamboo genome. A dendrogram was constructed which classified bamboo species into two major groups which coincided with rhizome type, runner, and clumper. The results of this study demonstrate that rice SSR markers can be a valuable source of markers for those genomes lacking useful marker systems.

Another extended application of microsatellite markers is mining of SSR markers from Expressed Sequence Tags. Ramalakshmi *et al.* (2010) examined a total of 3419 EST sequences from three bamboo species, namely, *Phyllostachys edulis*, *Bambusa oldhamii* and *Dendrocalamus sinicus* for the presence of di to hexa microsatellites. The frequency of SSR containing ESTs varied from 5.36% in *B. oldhamii* to 13.05% in *P. edulis*. No SSRs were found
in *D. sinicus*. Tri-nucleotide repeats (49.34%) were most frequent in *P. edulis*, while not much comparable difference in repeats was found in *B. oldhamii*. Flanking primer pairs were also designed in-silico for the sequences containing SSRs and their position on the genome hypothesized using similarity searching. SSRs located in open reading frame (ORF) were given functional annotation using Gene Ontology. Polymorphic SSRs were also detected using new pipeline- poly SSR. Polymorphism level was very low (2.43%).

Combination of different molecular markers for determining the genetic diversity among the considered populations has also been done. A study was performed on assessment of genetic relationships among 22 taxa of bamboo revealed by ISSR and EST-based random primers by Mukherjee *et al.* (2010). They evaluated 22 taxa of bamboo using 12 inter simple sequence repeats (ISSR) and four expressed sequence tag (EST)-based random primers, resulting in amplification of 220 loci. The grouping of species based on Jaccard's similarity matrix using UPGMA and principal coordinate analysis mostly agreed with earlier molecular phylogenetic studies in bamboo. However, species of one genus were placed in different clusters along with members of other genera. This calls for correct taxonomic delineation at the genus and species level using both vegetative and reproductive characters and correlation of molecular data with morphologically definable taxonomic groupings at the proper taxonomic level.

Such an approach was made by developing microsatellite markers for *Aulonemia aristulata* (*Poaceae*) and cross-amplification in other 18 bamboo species by Abreu *et al.* (2011). Using an enrichment genomic library, 13 microsatellite loci were isolated and characterized in *A. aristulata*. Seven of these loci were polymorphic. Twelve markers were cross-amplified in at least ten of the tested bamboo species.
Yong-Feng et al. (2011) analyzed genetic variation of some bamboo species by employing three different DNA markers viz. AFLP, inter-simple sequence repeats (ISSR) and sequence-related amplified polymorphism (SRAP). AFLP and ISSR were used to analyze genetic variations among 12 infra-species of 4 species of *Bambusa*, with 501 and 171 bands generated using 12 AFLP primer pairs and 10 ISSR primers, respectively, of which polymorphic loci accounted for 73.6% (367 loci) and 78.8% (137), respectively. It was revealed by two markers that there were abundant genetic variations among different bamboo species but few genetic variations among different infra-species. Similar results were obtained by AFLP, ISSR and (SRAP) analysis of 12 infra-species of 4 species of *Phyllostachys* and *Pseudosasa*.

Waghmare et al. (2013) attempted genetic characterization by PCR-RAPD of four important species of bamboo, found in Sindhudurg district, Maharashtra, viz. *Bambusa bambos*; *Pseudoxytenanthera ritcheyi*; *Pseudoxytenanthera stocksii* and *Dendrocalamus strictus*.

Similar approach was made using combination of AFLP and RAPD to study genetic diversity assessment of industrially important reed bamboo by Nag et al. (2013). Study was conducted using randomly collected industrially important reed bamboos (*O. travancorica*) from Kerala region of India. Fifty primers (8 AFLP and 42 RAPD) detected 914 polymorphic loci. Cluster and Principal Coordinate Analysis (PCA) based on combined AFLP and RAPD data grouped all the random accessions into three different populations. AMOVA revealed a moderate to high level of genetic variation. They reported that most of the variation occurred within population (54%) than among population (46%). Highly significant and high PhiPT estimate (Fst value; 0.456) indicated that these populations are not panmictic and are significantly isolated.
2.7 DNA BARCODING

Hebert et al. (2003b) established that the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core of a global bio-identification system for animals. First, they demonstrated that COI profiles, derived from the low-density sampling of higher taxonomic categories, ordinarily assign newly analyzed taxa to the appropriate phylum or order. Second, they demonstrated that species-level assignments can be obtained by creating comprehensive COI profiles. A model COI profile, based upon the analysis of a single individual from each of 200 closely allied species of lepidopterans, was 100% successful in correctly identifying subsequent specimens.

Kress et al. (2005) proposed the nuclear internal transcribed spacer region and the plastid trnH-psbA intergenic spacer as potentially usable DNA regions for applying barcoding to flowering plants. They compared the total plastid genomes of tobacco and deadly nightshade obtained from closely related species in seven plant families and a group of species sampled from a local flora encompassing 50 plant families (for a total of 99 species, 80 genera, and 53 families), and suggested that the sequences in this pair of loci have the potential to discriminate among the largest number of plant species for barcoding purposes.

Hajibabaei et al. (2005) studied the use of DNA barcodes, from a standardized region of the genome, to be used as a tool to facilitate species identification and discovery. In this study they showed that cytochrome c oxidase I DNA barcodes effectively discriminate among species in three Lepidoptera families from Area de Conservacion Guanacaste in Northwestern Costa Rica. They found that 97.9% of the 521 species recognized by prior taxonomic work possess distinctive cytochrome c oxidase I barcodes and that the few instances of interspecific sequence overlap involve very similar species.
Druzhinina et al. (2005) provided a convenient, on-line method for the quick molecular identification of Hypocre/Trichoderma at the genus and species levels based on an oligonucleotide barcode: a diagnostic combination of several oligonucleotides (hallmarks) specifically allocated within the internal transcribed spacer 1 and 2 (ITS1 and 2) sequences of the rDNA repeat. The barcode was developed on the basis of 979 sequences of 88 vouchered species which displayed in total 135 ITS1 and 2 haplotypes. Oligonucleotide sequences which are constant in all known ITS1 and 2 of Hypocre/Trichoderma but divergent in closely related fungal genera were used to define genus-specific hallmarks. The library of species-, clade- and genus-specific hallmarks is stored in the MySQL database and integrated in the TrichOKey v. 1.0 - barcode sequence identification program with the web interface located on www.isth.info. TrichOKey v. 1.0 identifies 75 single species, 5 species pairs and 1 species triplet. Verification of the DNA-barcode was done by a blind test on 53 unknown isolates of Trichoderma, collected in Central and South America. The obtained results were in a total agreement with phylogenetic identification based on tef1 (large intron), NCBI BLAST of vouchered records and postum morphological analysis. They concluded that oligonucleotide barcode is a powerful tool for the routine identification of Hypocre/Trichoderma species and should be useful as a complement to traditional methods.

Armstrong and Ball (2005) used DNA barcodes for biosecurity from invasive species after proper identification. DNA of tussock moth and fruit fly specimens intercepted at the New Zealand border over the last decade were reanalyzed using the COXI sequence barcode approach. Species identifications were compared with the historical dataset obtained by PCR–RFLP of nuclear rDNA. There was 90 and 96% agreement between the methods for these
species, respectively. Improvements included previous tussock moth ‘unknowns’ being placed to family, genera or species and further resolution within fruit fly species complexes.

Sass et al. (2007) performed a study to test the utility of two alternatives, the chloroplast *psbA-trnH* intergenic spacer and the nuclear ribosomal internal transcribed spacer (nr*ITS*), were tested for their utility in generating unique identifiers for members of the Cycadales. Ease of amplification and sequence generation with universal primers and reaction conditions was determined for each of the seven proposed markers. While none of the proposed markers provided unique identifiers for all species tested, nr*ITS* showed the most promise in terms of variability.

A universal primer cocktail for fish DNA barcoding was developed by Ivanova et al. (2007) using *COI* gene with an effort to gather DNA barcodes for all fish species. In this study, the success of polymerase chain reaction amplification and the quality of resultant sequences using three primer cocktails on DNA extracts from representatives of 94 fish families were evaluated.

Bucklin et al. (2007) conducted a study to discriminate many species of euphausiids. They utilized 650 bp region of mitochondrial cytochrome oxidase I (mt*COI*) which was sequenced for 40 species of 10 euphausiid genera: *Bentheuphausia*, *Euphausia*, *Meganyctiphanes*, *Nematobrachion*, *Nematoscelis*, *Nyctiphanes*, *Stylocheiron*, *Tessarabrachion*, *Thyssanoessa* and *Thysanopoda*. mt*COI* sequence variation discriminated all species; pairwise differences averaged 16.4% (range 7–24%); mean generalized time reversible (GTR) genetic distance was 26.7%. mt*COI* reliably identified euphausiid species: variation within species was typically, 1% and GTR distance was typically, 2%. Atlantic and Pacific Ocean populations of *Euphausia brevis* differed by 13% (GTR genetic distance ¼ 28%) and may deserve status as
distinct species. mtCOI gene trees were reconstructed for five genera using maximum parsimony, maximum likelihood and Bayesian algorithms; best-fit models of nucleotide evolution were determined for each genus. The mtCOI gene tree for 20 species of Euphausia reproduced one of three morphologically defined species groups. mtCOI resolved relationships among closely related species of most genera, usually in accord with morphological groupings. A comprehensive DNA barcode database for euphausiids will help ensure accurate species identification, recognition of cryptic species and evaluation of taxonomically meaningful geographic variation.

Edwards et al. (2008) worked on testing the potential of three regions (the nuclear ribosomal ITS and the plastid psbA-trnH and trnT-trnL intergenic spacers) for DNA barcoding in the plant genus Aspalathus L. (Fabaceae: Crotalarieae). In the study 51-species dataset for the psbA-trnH and ITS regions, 45% and 16% of sequences respectively were identical to the sequence of at least one other species, with two species undiscriminated even when the two regions were combined. In contrast, trnT-trnL discriminated between all species in this dataset. In a larger ITS and trnT-trnL dataset, including a further 82 species, 7 species in five pairwise comparisons remained undiscriminated when the two regions were combined. Four of the five pairs of species not discriminated by sequence data were readily distinguished using a combination of qualitative and quantitative morphological data. In the case of psbA-trnH, three intraspecific samples had a sequence identical to at least one other species. Overall, psbA-trnH, currently a candidate for plant barcoding, was the least discriminatory region in our study.

Fazekas (2008) carried a study to compared eight candidate plant barcoding regions from the plastome and one from the mitochondrial genome for how well they discriminated the monophyly of 92 species in 32 diverse genera of land plants (N = 251 samples). The plastid
markers comprise portions of five coding (rpoB, rpoC1, rbcL, matK and 23S rDNA) and three non-coding (trnH-psbA, atpF-atpH, and psbK-psbI) loci. The study included several taxonomically complex groups, and in all cases they examined multiple populations and species. The regions differed in their ability to discriminate species, and in ease of retrieval, in terms of amplification and sequencing success. Single locus resolution ranged from 7% (23S rDNA) to 59% (trnH-psbA) of species with well-supported monophyly. Sequence recovery rates were related primarily to amplification success (85–100% for plastid loci), with matK requiring the greatest effort to achieve reasonable recovery (88% using 10 primer pairs). Several loci (matK, psbK–psbI, trnH-psbA) were problematic for generating fully bidirectional sequences. Combining the more variable plastid markers provided clear benefits for resolving species, although with diminishing returns, as all combinations assessed using four to seven regions had only marginally different success rates (69–71%; values that were approached by several two- and three-region combinations).

Rivera (2009) performed a study in which, two species were isolated from the intestinal tracts of caterpillars from Costa Rica, and two potential species complexes, P. sclerotiorum and P. oxalicum belonging to Penicillium subgenus Aspergilloides and Furcatum, were studied using the Genealogical Concordance Concept (GCC) recognition criterion and barcoding methods. Analyses with P-tubulin (BenA), the nuclear internal transcriber spacer (ITS) region, Coxl, translation elongation factor 1-a (TEFl-a), and calmodulin (CaM) revealed that the Penicillium species isolated from Costa Rica are undescribed, and that P. sclerotiorum is a complex of seven phylogenetic species (including the Costa Rican species) that fit the prevailing morphological concept of P. sclerotiorum. The phylogenetic species were compared and newly discovered diagnostic morphological characters were used to create a taxonomic key to the species of the
complex. The new species are formally described as *P. guanacastense*, *P. mallochii*, *P. krugii*, *P. cainii*, *P. jacksonii* and *P. ciebiessum*. Analyses of multiple strains of *P. oxalicum* revealed that it is a single phylogenetic species, despite having a world-wide distribution, an unusually high degree of morphological variation, and a diversity of ecological roles.


Ma *et al.* (2010) made an attempt to discriminate species by a standard DNA sequence with universal primers and sufficient variation in medicinal pteridophytes. To determine whether DNA barcoding would be effective for differentiating pteridophyte species, they first analyzed five DNA sequence markers (*psbA-trnH* intergenic region, *rbcL*, *rpoB*, *rpoC1*, and *matK*) using six chloroplast genomic sequences from GeneBank and found *psbA-trnH* intergenic region the best candidate for availability of universal primers. The *psbA-trnH* region from 79 samples of medicinal pteridophyte plants was amplified. They found that the sequence of the *psbA-trnH* intergenic region can be determined with both high polymerase chain reaction (PCR) amplification efficiency (94.1%) and high direct sequencing success rate (81.3%). Combined with GeneBank data (54 species cross 12 pteridophyte families), species discriminative power analysis showed that 90.2% of species could be separated/identified successfully by the TaxonGap method in conjunction with the Basic Local Alignment Search Tool 1 (BLAST1) method. The TaxonGap method results further showed that, for 37 out of 39 separable species
with at least two samples each, between-species variation was higher than the relevant within-species variation. Thus, the \textit{psbA-trnH} intergenic region is a suitable DNA marker for species identification in medicinal pteridophytes.

Saunders and Kucera (2010) evaluated the universality and species discriminatory power of the plastid rubisco large subunit (\textit{rbcL}) (considering 5’ and 3’ fragments independently), elongation factor \textit{tufA}, and universal amplicon (UPA), and the nuclear D2/D3 region of the large ribosomal subunit (LSU) and the internal transcribed spacer of the ribosomal cistron (\textit{ITS}) were evaluated for their utility as DNA barcode markers for green macroalgae. Excepting low success for \textit{ITS}, all of these markers failed for the Cladophoraceae. For the remaining taxa, the 3’ region of the \textit{rbcL} (\textit{rbcL-3P}) and \textit{tufA} had the largest barcode gaps (difference between maximum intra- and minimum inter-specific divergence). Moderate amplification success (80 % excluding \textit{Cladophoraceae}) caused, at least in part, by the presence of introns within the \textit{rbcL-3P} for some taxa reduced the utility of this marker as a universal barcode system. The \textit{tufA} marker, had strong amplification success (95% excluding the \textit{Cladophoraceae}) and no introns were uncovered. They recommend that \textit{tufA} be adopted as the standard marker for the routine barcoding of green marine macroalgae (excluding the \textit{Cladophoraceae}).

Peng \textit{et al.} (2010) reported the cloning and sequencing of 10,608 putative full length cDNAs (FL-cDNAs) primarily from Moso bamboo, \textit{Phyllostachys heterocycla} cv. pubescens, a large woody bamboo with the highest ecological and economic values of all bamboos. This represents the third largest FL-cDNA collection to date of all plant species, and provides the first insight into the gene and genome structures of bamboos. They developed a Moso bamboo genomic resource database that so far contained the sequences of 10,608 putative FL-cDNAs and nearly 38,000 expressed sequence tags (ESTs) generated in this study. They concluded that
analysis of FL-cDNA sequences showed that bamboo diverged from ITS close relatives such as rice, wheat, and barley through an adaptive radiation. A comparative analysis of the lignin biosynthesis pathway between bamboo and rice suggested that genes encoding caffeoyl-CoA O-methyltransferase may serve as targets for genetic manipulation of lignin content to reduce pollutants generated from bamboo pulping.

Pryer et al. (2010) exponentially utilized DNA barcoding technique to expose a case of mistaken identity in the fern horticultural trade. In the present study they provide an example of how DNA barcoding approaches can be useful to the horticultural community for keeping plants in the trade accurately identified. They used plastid rbcL, atpA, and trnG-R sequence data to demonstrate that a fern marketed as Cheilanthes wrightii in the horticultural trade is, in fact, Cheilanthes distans. They strongly advocated about the fact that the barcoding approach may prove itself a valuable new technology available to the horticulture industry to help correct plant identification errors in the international trade.

Ribak (2010) conducted a study to analyze mitochondrial DNA (mtDNA) barcoding’s potential to identify known species and provide a well-resolved phylogeny for the New Zealand cicada genus Kikihia. For the purpose he created a phylogenetic tree for species in the genus Kikihia based on the barcoding region and compared it to a phylogeny previously created by Marshall et al. (2008). The effect of sampling on the success of barcoding studies was also analyzed. Subsets of a larger, more densely sampled dataset for the Kikihia Muta Group was additionally analyzed to determine which aspects of sampling strategy led to the most accurate identifications. Two species (K. “murihikua” and K. angusta) that were known to hybridize were studied. Individuals that were not obvious hybrids (determined by morphology) were selected for
the case study. Phylogenetic analysis of the barcoding region revealed insights into the reasons these two species could not be successfully differentiated using barcoding alone.

Bafeel et al. (2011) performed a study to evaluate the success rates of universal primers for amplification of \textit{matK} and \textit{rbcL} loci in 26 different plant species (covering 14 families) from Saudi Arabia. Success rate in PCR was higher for \textit{rbcL} (88\%) compared with \textit{matK} (69\%). The universal primers of both \textit{matK} and \textit{rbcL} failed to amplify the DNA from 3 plant species belonging to the family \textit{Asteraceae} (\textit{Anthemis deserti, Pulicaria undulate, and Sonchus oleraceus}). Two plant species \textit{Malva parviflora} (\textit{Malvaceae}) and \textit{Salsola imbricate} (\textit{Chenopodiaceae}) indicated different primer binding site (\textit{matK}) as the amplified PCR products were of lower size than expected for these species. These findings indicate that although currently used universal primers of \textit{rbcL} and \textit{matK} are able to amplify many of the plant species they may fail in certain cases due to primer mismatch at the annealing site.

Cohen (2011) focused on the utility of three noncoding cpDNA regions (\textit{trnH-psbA, trnL-trnL-trnF}, and \textit{trnS-trnG-trnG}) at identifying species from the genus \textit{Prunus} L. Sequence data was generated for \textit{matK} and \textit{rbcL} using the same \textit{Prunus} taxa to determine how well these two regions would delimit species compared to the three noncoding cpDNA regions. In addition to this, sequence data for \textit{matK} and \textit{rbcL} were generated for 27 angiosperm taxa and compared to 34 previously tested noncoding chloroplast gene regions to determine their relative genetic variability. The study demonstrated that \textit{matK} and \textit{rbcL} contain less genetic variability than noncoding regions. Based on the number of potentially informative characters (PIC), \textit{matK} was the 25th most variable region and \textit{rbcL} was the 34th most variable region out of 36 regions tested. The finding of the study was that, no region alone or in combination was able to
discriminate > 50% of species, and noncoding cpDNA regions typically outperformed the
Consortium for the Barcode of Life’s combination of \( \text{matK} + \text{rbcL} \).

Stoeckle et al. (2011) tested recovery of standard DNA barcodes for land plants from a
large array of commercial tea products and analyze their performance in identifying tea
constituents using existing databases. Most (90%) of 146 tea products yielded \( \text{rbcL} \) or \( \text{matK} \)
barcodes using a standard protocol. About 1/3 of herbal teas generated DNA identifications not
found on labels.

Yu et al. (2011) evaluated the entire \( \text{matK} \) region to find a region of 600–800 bp that is
highly variable, and which would represents the best of all \( \text{matK} \) regions with priming sites
conservative enough to design universal primers, and avoids the mononucleotide repeats. They
chose the region in the middle and a pair of primers named \( \text{matK472F} \) and \( \text{matK1248R} \) was
designed to amplify and sequence the \( \text{matK} \) fragment of approximately 776 bp. The region
exhibited high amplification rates and quality of sequences. The universality of this primer pair
was tested using 58 species from 47 families of angiosperm plants. The primers showed a strong
amplification (93.1%) and sequencing (92.6%) successes in the species tested. They proposed
that the new primers will solve, in part, the problems encountered when using \( \text{matK} \) and promote
the adoption of \( \text{matK} \) as a DNA barcode for angiosperms.

Groot et al. (2011) evaluated the potential of a combination of \( \text{rbcL} \) with a noncoding
plastid marker, \( \text{trnL-F} \), to obtain DNA identifications for fern species. A regional approach was
adopted, by creating a reference database of trusted \( \text{rbcL} \) and \( \text{trnL-F} \) sequences for the wild-
occurring homosporous ferns of NW-Europe. A combination of parsimony analyses and distance
based analyses was performed to evaluate the discriminatory power of the two-region barcode.
86 tiny fern gametophytes were used for DNA extraction and were used as a test case for the
performance of DNA-based identification. Primer universality proved high for both markers. Based on the combined rbcL + trnL-F dataset, all genera as well as all species with non-equal chloroplast genomes formed their own well supported monophyletic clade, indicating a high discriminatory power. Interspecific distances were larger than intraspecific distances for all tested taxa. All test samples could be identified to genus level, species identification was well possible unless they belonged to a pair of Dryopteris species with completely identical chloroplast genomes. Their results suggested a high potential of the combined use of rbcL and trnL-F as a two-locus cpDNA barcode for identification of fern species.

Rajapakse et al. (2012) carried out a study to determine the species limits of the endemic genus Hortonia by DNA barcoding with a view to establish conclusive molecular evidence regarding the speciation of Hortonia in Sri Lanka. Total DNA was extracted from all three species. Internal transcribed spacer (ITS) region and the trnH-psbA region were first amplified with specific primers and ligated to a pBlueScript vector followed by plasmid purification. The purified plasmids containing the DNA of interest were subjected to sequencing. Sequence homology of ITS and trnH-psbA from all three species were compared using MacVector software. Between H. ovalifolia and H. floribunda, the ITS region showed a 2.37 % sequence divergence and the trnH-psbA region showed a 1.5 % sequence divergence. Between H. ovalifolia and H. angustifolia, the ITS region and trnH-psbA region showed a 3.36 % and 1.89 % sequence divergences, respectively. The percentage sequence divergence between ITS and trnH-psbA regions of H. floribunda and H. angustifolia were 3.36 % and 2.65 %, respectively. The high sequence divergence values clearly indicated that the genus Hortonia has three different species. Considering the percentage sequence divergence values, H. ovalifolia and H. floribunda were more closely related to each other than to H. angustifolia.
Smith et al. (2012) examined the extent, to which Wolbachia, an endosymbionts may be detected in routine DNA barcoding. They assessed their impact upon the insect sequence divergence and identification accuracy, and considered the variation present in Wolbachia COI. Using both standard PCR assays (Wolbachia surface coding protein – wsp), and bacterial COI fragments they found evidence of Wolbachia in insect total genomic extracts created for DNA barcoding library construction. 0.16% of Wolbachia COI was present on examining 0.2 million insect COI trace files available on the Barcode of Life Datasystem (BOLD).

Cai et al. (2012) performed a study for testing four candidate barcoding markers in temperate woody bamboos (Poaceae: Bambusoideae). They made an attempt to test the feasibility of four proposed DNA barcoding markers (matK, rbcL, trnH–psbA, and internal transcribed spacer [ITS]) in identifying 27 species of the temperate woody bamboos. Three plastid markers showed high levels of universality, whereas the universality of ITS was comparatively low. A single plastid marker provided low levels of discrimination success at both the genus and species levels (<12%). Among the combinations of plastid markers, the highest discriminatory power was obtained using the combination of rbcL + matK (14.8%). Using a combination of three markers did not increase species discrimination. The nuclear region ITS alone could identify 66.7% of species, although fewer taxa were included in the ITS analyses than in the plastid analyses. When ITS was integrated with a single or combination of plastid markers, the species discriminatory power was significantly improved. They suggested that a combination of rbcL + ITS, which exhibited the highest species identification power of all combinations in the present study, could be used as a potential DNA barcode for temperate woody bamboos.
Mankga (2012) targeting the most commonly used medicinal plants in South Africa and produced a set of barcodes for fast and easy DNA-based species identification (*rbcL* & *matK*). The efficiency of core barcodes was tested for the identification of medicinal plants using four main analyses, in the R package Spider 1.1-1. He examined the extent of specific genetic divergence, DNA barcoding gap, BLAST test, and the ability to discriminate between species were assessed. Overall, the *matK* region was found to be a more useful tool for the species identification of medicinal plants in South Africa.

Schoch *et al.* (2012) studied the nuclear ribosomal internal transcribed spacer (*ITS*) region for *ITS* usage as a universal DNA barcode marker for fungi. Three subunits from the nuclear ribosomal RNA cistron were compared together with regions of three representative protein coding genes (largest subunit of RNA polymerase II, second largest subunit of RNA polymerase II, and minichromosome maintenance protein). Among the regions of the ribosomal cistron, the internal transcribed spacer (*ITS*) region has the highest probability of successful identification for the broadest range of fungi, with the most clearly defined barcode gap between inter- and intraspecific variation. The nuclear ribosomal small subunit has poor species-level resolution in fungi. They formally proposed *ITS* as the primary fungal barcode marker to the Consortium for the Barcode of Life, with the possibility that supplementary barcodes may be developed for particular narrowly circumscribed taxonomic groups.

Boswell (2013) discovered one PCR-RFLP marker (*trnT/D* digested with Alu1) and two DNA barcoding regions (*matK* and *trnL-F*) to distinguish between cpDNA haplotypes for yellow toadflax and Dalmatian toadflax. Testing on individual plants collected from multiple U.S. field hybridization sites has revealed that yellow toadflax chloroplast DNA occurs more frequently in
hybrids than Dalmatian toadflax cytoplasm. These results indicated that gene flow is asymmetric in persistent *L. vulgaris* x *L. dalmatica* populations.

Sosa *et al.* (2013) worked on the applications of DNA barcoding for the identification of illegally traded endangered species from small samples or vegetative specimens. They created a DNA barcode library for 20 endangered Orchidaceae species and 36 species of bamboo (Bambusoideae, Poaceae) distributed in Mexico. They applied several metrics to evaluate the efficiency of the barcodes *matK* and *rbcL* and, for bamboos, that of the plastid spacer psbl-K.

Gere *et al.* (2013) performed a study in which they focused on how the addition of complementary barcodes (*nrITS* and *trnH-psbA*) to the core barcodes will affect the performance of the core barcodes in discriminating closely related species from family to section levels. They found that the core barcodes performed poorly compared to the various combinations tested. Using multiple criteria, they finally advocated for the use of the core + *trnH-psbA* as potential DNA barcode for the family *Combretaceae* at least in southern Africa. Their results also indicated that the success of DNA barcoding in discriminating closely related species may be related to evolutionary and possibly the biogeographic histories of the taxonomic group tested.

Shalini *et al.* (2013) investigated identification of genetic relationships in 10 species of bamboo using random amplified polymorphic DNA (RAPD) technique and their association with morphological characters was also studied. Analysis started by using twenty five markers (Twenty one RAPD and four ISSR) that were used to distinguish 10 species. Out of twenty five, ten primers were used for identification and for establishing a profiling system to estimate genetic diversity. A total of three hundred ninety alleles were amplified out of this hundred distinct polymorphic DNA fragments (bands), ranging from 1.0–0.2 kb were amplified by 10 selected primers. On the basis of morphological traits, *Bambusa tulda* and *Bambusa balcoa* were
very similar to each other for all four characters. While Guadua angustifolia showed very different types of character in all four traits but it showed highest number of new culms as compared to others. The genetic similarity analysis was conducted based on presence or absence of bands, which revealed a wide range of variability among the species. Cluster analysis clearly showed two major clusters belonging to 10 species of bamboo. Major Cluster1 was further subdivided into three minor clusters. The species of Bambusa tulda and Bambusa balcoa were the most closely related and formed the third minor cluster along with Bambusa nutans and Dendrocalamus strictus. The variety Guadua angustifolia was very distinct and showed as an out group in the dendrogram that was single species in cluster B. Thus, the morphological characterization was confirmed by the genetic diversity analysis.

Li et al. (2013) tested whether the internal transcribed spacer 2 (ITS2) region is an effective marker for using in authenticating of the Schisandra chinensis at the species and population levels, separately. And the results showed that the wild populations had higher percentage of individuals that had substitution of C→A at site 86-bp than the cultivated populations. At sites 10-bp, 37-bp, 42-bp and 235-bp, these bases of the Schisandra sphenanthera samples differed from that of S. chinensis. Two species showed higher levels of inter-specific divergence than intra-specific divergence within ITS2 sequences. However, 24 populations did not demonstrate much difference as inter-specific and intra-specific divergences were concerned. Both S. chinensis and S. sphenanthera showed monophyly at species level, yet the samples of different populations shown polyphyly at population level. ITS2 performed well when using BLAST1 method. ITS2 obtained 100% identification success rates at the species level for S. chinensis, with no ambiguous identification at the genus level for ITS2 alone. The
ITS2 region could be used to identify *S. chinensis* and *S. sphenanthera* in the “Chinese Pharmacopoeia”.

Saarela *et al.* (2013) performed a study to facilitate rapid identification and accurate identification of Arctic plant species. They generated DNA barcodes for the core plastid barcode loci (*rbcL* and *matK*) for 490 vascular plant species, representing nearly half of the Canadian Arctic flora and 93% of the flora of the Canadian Arctic Archipelago. Sequence recovery was higher for *rbcL* than *matK* (93% and 81%), and *rbcL* was easier to recover than *matK* from herbarium specimens (92% and 77%). Distance-based and sequence similarity analyses of combined *rbcL* + *matK* data discriminate 97% of genera, 56% of species, and 7% of infraspecific taxa. They even characterize barcode variation in detail in the ten largest genera sampled (*Carex, Draba, Festuca, Pedicularis, Poa, Potentilla, Puccinellia, Ranunculus, Salix, and Saxifraga*) in the context of their phylogenetic relationships and taxonomy. Discrimination with the core barcode loci in these genera ranges from 0% in *Salix* to 85% in *Carex*. Of three supplementary barcode loci (*psbA–trnH, psbK–psbI, atpF–atpH*) collected for a subset of *Poa* and *Puccinellia* species, only *atpF–atpH* improved discrimination in *Puccinellia*, compared with *rbcL* and *matK*. Variation in *matK* in *Vaccinium uliginosum* and *rbcL* in *Saxifraga oppositifolia* corresponds to variation in other loci used to characterize the phylogeographic histories of these Arctic-alpine species.

Kelchner, along with the Bamboo Phylogeny Group (2013), presented the most complete phylogeny estimation to date of bamboo tribes and subtribes using 6.7 kb of coding and noncoding sequence data and 37 microstructural characters from the chloroplast genome. Quality of data was assessed. Four major plastid lineages were recognized: temperate woody, paleotropical woody, neotropical woody, and herbaceous bamboos. Woody bamboos were
resolved as paraphyletic with respect to *Olyreae* but SH tests cannot reject monophyly of woody species (*Arundinarioideae* + *Bambuseae*).

In another study Dong *et al.* (2013) presented the chloroplast genome of *Sedum sarmentosum* and with the help of several available (or elucidated) chloroplast genomes, examined the evolution of chloroplast genomes in *Saxifragales*. The chloroplast genome of *S. sarmentosum* is 150,448 bp long and includes 82,212 bp of a large single-copy (LSC) region, 16,670 bp of a small single-copy (SSC) region, and a pair of 25,783 bp sequences of inverted repeats (IRs). The genome contains 131 unique genes, 18 of which were duplicated within the IRs. Based on a comparative analysis of chloroplast genomes from four representative *Saxifragales* families, they observed two gene losses and two pseudogenes in *Paeonia obovata*, and the loss of an intron was detected in the *rps16* gene of *Penthorum chinense*. Comparisons among the 72 common protein-coding genes confirmed that the chloroplast genomes of *S. sarmentosum* and *Paeonia obovata* exhibit accelerated sequence evolution. In addition, a strong correlation was observed between the rates of genome evolution and genome size. The genome sizes of these species were negatively correlated with nucleotide substitution rates.

Ogedengbe (2015) tested utility of mt *COI* sequences for identification and phylogenetics of tissue (i.e. *Sarcocystidae*), eimeriid (e.g. *Eimeriidae*, *Lankesterellidae*) and adeleid coccidia (i.e. *Hepatozoon* and other *haemogregarines*). Degenerate primers of broad specificities generated mt *COI* DNA barcodes that, with few exceptions (notably *Cystoisospora* spp.), identified any enteric (e.g. *Isospora*, *Cyclospora*, *Eimeria*) or tissue (e.g. *Toxoplasma*, *Hammondia*, *Sarcocystis*) coccidium. The mt *COI* locus was recommended as a suitable DNA barcoding target for coccidian parasites of veterinary and zoonotic importance. Complete mt genomes of >20 *eimeriid* and *adeleid coccidia* were sequenced. More 17 species representing 6
genera of eimeriid (Eimeria, Isospora, Caryospora, Cyclospora, Lankesterella) and adeleid coccidia (Hepatozoon catesbiana) were sequenced. Phylogenetic analyses using mt CDS sequences (i.e. partial mt COI or concatenated CDS sequences) with or without nuclear 18S rDNA sequences suggest combined nu 18S rDNA and mt COI sequences (analysed using partitioned data with suitable nucleotide substitution models for each partition) provide robust evolutionary hypotheses for these parasites.

Jian et al. (2014) used DNA barcoding techniques to explore the composition of roasted barley tea (Hordeum vulgare), which was exported to X country from China but then returned, due to the detection of other plant components present. They amplified four commonly used DNA barcodes (rbcL, matK, trnH-psbA, ITS2) to identify the plant components in the roasted barley tea. Out of the 13 batches, one (7.7%) was substituted with Morus sp.; twelve (92.31%) contained authentic with H. vulgare DNA barcodes. Out of the 12 authentic batches only two (15.38%) were detected with only H. vulgare DNA barcodes, whilst ten (76.9%) were contaminated with other plant components.

Yu et al. (2014) performed a study to determine the efficiency of expedient identification in Magnoliaceae species by DNA barcoding. In the study, they collected 83 samples belonging to 68 species in 10 genera of Magnoliaceae. Candidate DNA regions (i.e., psbA-trnH, matK, rbcL, ITS, ITS2, rpoB, and rpoC1) were amplified and sequenced for the evaluation of their PCR amplification, sequencing efficiency, intra- and inter-specific divergence and barcoding gap by sequence alignment and Kimura 2-Parameter (K2P) distance analysis, and the rate of correct identification was assessed by BLAST analysis. The results showed that psbA-trnH and matK exhibited high performance in efficiency of PCR amplification and the rate of successful sequencing, followed by rbcL. Associated with the analysis of199 sequences for 96 species in 9
genera of Magnoliaceae retrieved from GenBank, it was discovered that psbA-trnH was highest in inter-specific divergence and rate of correct identification, indicating ITS efficiency in the identification of Magnoliaceae species. Besides, matK was also easy to amplify and had high rate of correct identification, suggesting ITS potential to distinguish Magnoliaceae species. This study indicates that DNA barcoding provides an effective technique for the expedient identification of morphologically similar species, and it is a powerful aid to the conventional methods for species identification.

Li et al. (2014) conducted a study to analyze the classification and diversity of Auricularia auricula-judae. For this purpose, the molecular diversity of 32 A. auricula-judae commercial cultivars in China was analyzed by using the nuclear ribosomal DNA intergenic spacer. The complete nuclear rDNA gene complex of A. auricula-judae isolate is 11,210 bp long, and contains the 18S, 5.8S, and 28S rRNA gene as well as the ITS and IGS regions. Based on the sequence data, four more effective primer combinations for the IGS region of A. auricula-judae were designed. Nucleotide sequence variation in the IGS among 32 A. auricula-judae commercial cultivars in China sorted into three strongly supported clades, which is correlated with geographical regions. This work suggested that the IGS region can be used as an excellent tool for identification of genetic variation.

Steel et al. (2015) selected a total of 10 native plant specimens located in the campus of the Bronx Community College of New York City. They optimized the protocols provided by the CSHL to achieve DNA purification, rbcL amplification and sequencing. They applied bioinformatic tools (sequence alignment; substitution rate and time computation; 3D structure comparison) for DNA-based species identification, protein structure homology modeling and phylogenetic analysis.
Lien et al. (2015) in their study, they investigated the genetic diversity of soft corals in Nha Trang bay based on 696bp of msh1 gene and 866bp of irg-coxl fragments. The result indicated that eleven studied specimens were grouped into four different clades including Sinularia, Sarcophyton, Lobophytum and a mixed with Sarcophyton and Lobophytum genera. Both msh1 and irg-coxl characterized some specific substitutions in each clade to distinguish them from each other.

Kashmeera and Sudhikumar (2015) worked on barcoding of spider using mt DNA. The utility of DNA barcoding in identifying spider species was revealed in their study. The study established that the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core of a global bioidentification system for animals. The study also demonstrated that COI identification system will provide a reliable, cost-effective and accessible solution to the current problem of species identification. A comparative study of COI sequence of nine families of spiders and their phylogenetic analysis was performed and it showed their relationship with evolution of web building behavior. Thus this study revealed the relationship between molecular and morphological taxonomy.

Giudicelli et al., 2015, tested the performance of plastid and nuclear regions as DNA barcodes in Passiflora. They analyzed 1034 accessions of 222 species representing the four subgenera of Passiflora and evaluated the effectiveness of five plastid regions and three nuclear datasets currently employed as DNA barcodes in plants using barcoding gap, applied similarity-, and tree-based methods. The plastid regions were able to identify less than 45% of species, whereas the nuclear datasets were efficient for more than 50% using “best match” and “best close match” methods of TaxonDNA software. The nuclear ribosomal internal transcribed spacer
1 (*ITS1*) region presented a higher discrimination power than the other datasets and also showed other desirable characteristics as a DNA barcode for this genus.

The review of literature shows that manifold work has been conducted by different researchers in an attempt to fulfill the goal of bamboo identification using various tools at the biochemical level as well as at the molecular level. Classical taxonomy still plays an important role in classifying different species of bamboo. However, the molecular approaches such as DNA barcoding can definitely assist the traditional approaches and will make identification procedure much more authentic and easier.
3.1 Thirty four species of bamboo were selected for the present study

Plant material for the DNA extraction were collected from bambusetum, IFP, Ranchi, Jharkhand, India (Table No. 3.1)

Table 3.1. Thirty four species of bamboo used in the study

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Name of the Genus</th>
<th>Name of the species</th>
<th>Division according to Gamble, 1896 (VII)</th>
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<td>Bambusa bambos Bennet and Gaur</td>
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<td>3</td>
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<td>Bambusa multiplex (Lour.) Raeusch. ex Schult.f.</td>
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<td></td>
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<td>Bambusa polymorpha Munro</td>
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<td><em>Thyrsostachys siamensis</em> Gamble</td>
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3.2 Characteristics of the bamboo species studied

Descriptions of the different bamboo species used in the study (Fig 3.1):

1. *Bambusa balcooa* Roxb.

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1600–2000 cm long; 75–150 mm diam.; woody; without nodal roots. Culm-internodes terete; with small lumen; 40–45 cm long; dark green. Culm-nodes swollen; pubescent. Culm-sheaths 25–35 cm long; 1.1 times as long as wide; hairy on margins; truncate at apex; without auricles; ciliate on shoulders. Culm-sheath blade triangular; 15–20 cm long; pubescent. Leaves cauline. Leaf-sheaths striately veined; pubescent; hairs white.

2. *Bambusa bambos* Bennet and Gaur

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 2000–3000 cm long; 100–150 mm diam.; woody; with root thorns from the nodes. Culm-nodes pubescent (brown). Culm-sheaths deciduous; glabrous. Culm-sheath ligule 1–2 mm high; ciliolate. Culm-sheath blade triangular; erect; hispid; acute. Leaf-sheaths glabrous on surface; outer margin hairy. Leaf-sheath oral hairs setose; 4–6 mm long; pale. Leaf-sheath auricles erect.

3. *Bambusa multiplex* (Lour.) Raeusch. ex Schult.f.

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 200–400 cm long; 10–30 mm diam.; woody; without nodal roots. Culm-sheaths deciduous; auriculate; ciliate on shoulders. Culm-sheath ligule 1–1.5 mm high. Culm-sheath blade triangular; 1–2 cm long; acute. Leaf-sheath oral hairs setose; 3–5 mm long. Leaf-sheath auricles falcate.

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 200–400 cm long; 10–30 mm diam.; woody; without nodal roots. Culm-sheaths deciduous; auriculate; ciliate on shoulders. Culm-sheath ligule 1–1.5 mm high. Culm-sheath blade triangular; 1–2 cm long; acute. Leaves Leaf-sheath oral hairs setose; 3–5 mm long. Leaf-sheath auricles falcate.

5. *Bambusa nutans* Wall. Ex Munro

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 600–1200 cm long; 40–70 mm diam.; woody; with aerial roots from the nodes. Culm-nodes glabrous, or pubescent. Lateral branches dendroid. Culm-sheaths 15–23 cm long; pubescent; with appressed hairs; with black hairs; truncate at apex; auriculate; setose on shoulders. Culm-sheath ligule 2.5–5 mm high; dentate. Culm-sheath blade triangular; 15–23 cm long; pubescent; acute. Leaf-sheaths striately veined; pubescent. Leaf-sheath oral hairs setose. Leaf-sheath auricles falcate.

6. *Bambusa pallid* Munro

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1500–2000 cm long; 25–35 mm diam.; woody; without nodal roots. Culm-sheaths 20–30 cm long; 1 times as long as wide; pubescent; hairy throughout; with appressed hairs; truncate at apex; without auricles, or auriculate; setose on shoulders. Culm-sheath blade triangular; as wide as sheath at base; 20–35 cm long; pubescent; acuminate. Leaf-sheaths straightly veined; glabrous on surface. Leaf-sheath oral hairs setose; deciduous. Leaf-sheath auricles erect.
7. *Bambusa polymorpha* Munro

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1500–2500 cm long; 75–150 mm diam.; woody; with aerial roots from the nodes. Culm-nodes swollen. Culm-sheath blade triangular; cordate; 7.5 cm long; pubescent. Leaf-sheaths keeled; striately veined; pubescent. Leaf-sheath oral hairs scanty; deciduous. Leaf-sheath auricles falcate.


A tufted bamboo introduced in Orissa planted in Garden. 8-10 m high, 4-7 cm in diameter, smooth yellow with light green stripes or at times light green with yellow stripes; internodes 15-25 cm long, thick-walled. Culm sheaths: similar to B. vulgaris but more yellowish and little smaller. leaf-sheath smooth, truncate, ciliate. Young shoots are yellowish-brown, streaked; sheath with dark-brown appressed hairs, auricles distinctly falcate, blades yellowish-green to green.

9. *Bambusa tulda* Roxb

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 600–2000 cm long; 50–100 mm diam.; woody; without nodal roots. Culm-sheaths 15–23 cm long; 1 times as long as wide; pubescent; with appressed hairs; with tawny hairs; truncate at apex, or convex at apex; auriculate; ciliate on shoulders. Culm-sheath blade triangular; cordate; erect. Leaf-sheaths striately veined; glabrous on surface. Leaf-sheath oral hairs setose; pale. Leaf-sheath auricles falcate.

10. *Bambusa vulgaris* Schrad. ex Wendl.

Perennial; caespitose. Rhizomes short; pachymorph. Culms geniculately ascending; 1500–2000 cm long; 40–100 mm diam.; woody; without nodal roots. Culm-internodes terete; hollow. Culm-sheaths deciduous; hispid; with dark brown hairs; auriculate; ciliate.

11. *Bambusa wamin* Camus

*Bambusa vulgaris 'Wamin'* prefers full sun to partial shade. If grown in deep shade, the nodes won't be as pronounced, as the plant will stretch to reach for the sun. Although the roots are cold hardy, the foliage is frost sensitive and culm damage can result from temperatures below -2°C. Culms: This species is a small sized graceful bamboo. Culms are loosely tufted, dark shiny green, woody and thick-walled, with an average height between 2-5 m. Internodes are 10-15 cm long of which the lower internodes are shortened and swollen with nodal roots. Lower swollen internodes can be 10-12 cm in diameter. Seeds: Data on flowering cycles and seed setting is unknown.

12. *Cephalostachyum capitatum* Munro

Perennial; caespitose. Rhizomes short; pachymorph. Culms leaning, or scandent; 400–1000 cm long; 25–30 mm diam.; woody. Culm-internodes terete; hollow; 60–100 cm long; yellow, or mid-green. Culm-sheaths 15–30 cm long; 3–4 times as long as wide; chartaceous; pubescent; with appressed hairs; with tawny hairs; concave at apex, or truncate at apex; auriculate. Culm-sheath ligule entire, or ciliate. Culm-sheath blade lanceolate; erect, or reflexed; pubescent. Leaf-sheaths glabrous on surface. Leaf-sheath oral hairs scanty; deciduous.

13. *Dendrocalamus asper* (Schult.) Backer

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1500–2000 cm long; 120 mm diam.; woody; with aerial roots from the nodes. Culm-internodes terete; 40–50
cm long; distally pubescent. Culm-sheaths deciduous; 40–50 cm long; pale green; pubescent; with dark brown hairs; auriculate; with 7 mm high auricles; ciliate on shoulders; shoulders with 5 mm long hairs. Culm-sheath ligule 7–10 mm high; fimbriate. Culm-sheath blade linear; narrower than sheath; reflexed; 25 cm long; 35 mm wide; acuminate. Leaf-sheath oral hairs lacking.

14. *Dendrocalamus brandisii* (Munro) Kurz

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1900–3300 cm long; 130–200 mm diam.; woody; with aerial roots from the nodes. Culm-internodes terete; with small lumen; 30–38 cm long; grey. Culm-sheaths 40–60 cm long; 2 times as long as wide; coriaceous; pubescent; with white hairs; concave at apex; auriculate. Culm-sheath ligule 10–20 mm high; lacerate. Culm-sheath blade lanceolate; reflexed; 15–46 cm long; 80–130 mm wide; pubescent. Leaf-sheaths striately veined; pubescent. Leaf-sheath oral hairs scanty; deciduous.


Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 2000–3000 cm long; 200–300 mm diam.; woody. Culm-internodes terete; hollow; 30–45 cm long. Culm-sheaths deciduous; coriaceous; purple; pubescent; hairy throughout; with dark brown hairs; auriculate; glabrous on shoulders. Culm-sheath ligule 6–12 mm high; ciliate and dentate. Culm-sheath blade lanceolate; spreading.

16. *Dendrocalamus hamiltonii* Nees et Arn. ex Munro

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect, or arching; 1200–2000 cm long; 100–185 mm diam.; woody; with root dots on the nodes. Culm-internodes terete; with small lumen; 30–50 cm long; dark green, or grey; distally pubescent. Culm-
sheaths 35–45 cm long; 2 times as long as wide; antrorsely scabrous; glabrous, or hispid; with dark brown hairs; truncate at apex. Culm-sheath ligule entire. Culm-sheath blade lanceolate, or narrowly ovate; narrower than sheath; 15–30 cm long.

17. *Dendrocalamus membranaceus* Munro

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1000–1800 cm long; 60–100 mm diam.; woody. Culm-internodes terete. Culm-sheaths 7–15 cm long; coriaceous; hispid; with dark brown hairs; auriculate; with 10 mm high auricles; with 20 mm wide auricles; setose on shoulders; shoulders with 10–15 mm long hairs. Culm-sheath ligule ciliolate. Culm-sheath blade ovate; 2.5–3.5 cm long; acuminate. Leaf-sheaths hispid. Leaf-sheath oral hairs setose; 10 mm long. Leaf-sheath auricles falcate.

18. *Dendrocalamus sikkimensis* Gamble

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1700–2000 cm long; 120–200 mm diam.; woody. Culm-internodes terete; with small lumen; 30–45 cm long; dark green; antrorsely scabrous. Culm-sheaths 30 cm long; 0.9 times as long as wide; pubescent; with dark brown hairs; auriculate; setose on shoulders. Culm-sheath ligule dentate. Culm-sheath blade lanceolate; reflexed; 20–30 cm long; 50–70 mm wide. Leaf-sheaths smooth. Leaf-sheath oral hairs setose. Leaf-sheath auricles falcate

19. *Dendrocalamus strictus* (Roxb.) Nees

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 600–1500 cm long; 25–75 mm diam.; woody; without nodal roots, or with prop roots. Culm-internodes terete; solid; 30–45 cm long. Culm-nodes swollen. Culm-sheaths deciduous; 0.25–0.66 length of internode; coriaceous; hispid; hairy throughout; with dark brown hairs; auriculate; ciliate on shoulders. Culm-sheath blade lanceolate; acuminate.
20. *Dinochloa andamanica* Kurz


21. *Dinochloa macclellandii* (Munro) Kurz

Perennial; caespitose. Rhizomes short; pachymorph. Culms scandent; zigzag; 3000 cm long; 25–50 mm diam.; woody. Culm-internodes terete; hollow; 15–20 cm long; antrorsely scabrous; distally hispid. Culm-nodes swollen. Culm-sheaths persistent; 15–22 cm long; 1 times as long as wide; coriaceous; pubescent; hairy throughout; with appressed hairs; with tawny hairs; truncate at apex. Culm-sheath ligule 0.5 mm high; entire, or dentate. Culm-sheath blade narrowly ovate; spreading, or reflexed; 15–30 cm long; 25–60 mm wide; pubescent. Leaf-sheaths keeled; striately veined; glabrous on surface, or pubescent. Leaf-sheath oral hairs scanty. Leaf-sheath auricles falcate.

22. *Gigantochloa atroviolacea* Widjaja

Perennial; caespitose; clumped loosely. Rhizomes short; pachymorph. Culms erect; 600–1200 cm long; 60–80 mm diam.; woody; with aerial roots from the nodes. Culm-internodes terete; hollow; 40–50 cm long; purple; distally pubescent. Culm-sheaths deciduous; 16–20 cm long; hispid; with appressed hairs; with dark brown hairs; auriculate; with 3–5 mm high auricles; ciliate on shoulders; shoulders with 3–7 mm long hairs. Culm-sheath ligule 2 mm high; dentate. Culm-sheath blade ovate; spreading, or
reflexed; 4–9 cm long. Leaf-sheaths pubescent; hairs white. Leaf-sheath auricles erect; 1 mm long.

23. *Gigantochloa macrostachya* Kurz

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1000–1600 cm long; 6–10 mm diam.; woody. Culm-internodes terete; hollow; 40–80 cm long; dark green, or glaucous. Culm-nodes pubescent. Culm-sheaths 12–20 cm long; 0.5 times as long as wide; hispid; with appressed hairs; with black hairs; hairy on margins; truncate at apex; auriculate; ciliate on shoulders. Culm-sheath ligule entire, or dentate. Culm-sheath blade ovate; narrower than sheath; pubescent; acute. Leaf-sheaths keeled; pubescent. Leaf-sheath oral hairs scanty; deciduous. Leaf-sheath auricles falcate.

24. *Gigantochloa nigrociliata* (Buse) Kurz

Perennial; caespitose; clumped loosely. Rhizomes short; pachymorph. Culms erect; 1500–2000 cm long; 30–60 mm diam.; woody. Culm-internodes terete; hollow; 20–35 cm long; light green; distally pubescent. Culm-sheaths tardily deciduous; 11–18.5 cm long; hispid; with appressed hairs; with dark brown hairs; auriculate; with 2–4 mm high auricles. Culm-sheath ligule 2–3 mm high; dentate. Culm-sheath blade triangular; erect, or spreading; 6–10 cm long; 20–35 mm wide; acute. Leaf-sheath auricles erect; 1 mm long.

25. *Guadua angustifolia* Kunth

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 2000–3000 cm long; 100–130 mm diam.; woody; with root thorns from the nodes. Culm-internodes terete; hollow; 20 cm long. Culm-sheaths pubescent; hairy throughout; with dark brown hairs;
without auricles. Culm-sheath blade triangular; erect; pubescent. Leaf-sheath oral hairs lacking.

26. *Melocanna baccifera* (Roxb.) Kurz

Perennial; culms solitary. Rhizomes elongated; pachymorph. Culms erect; 1000–2000 cm long; 50–90 mm diam.; woody. Culm-internodes terete; hollow; 30–60 cm long. Culm-sheaths persistent; coriaceous; pubescent; hairy throughout; with yellow hairs; without auricles. Culm-sheath blade linear; reflexed. Leaf-sheath oral hairs lacking.

27. *Ochlandra scriptoria* (Dennst.) Fischer

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 300–500 cm long; 25 mm diam.; woody. Culm-internodes terete; hollow; 45 cm long; smooth. Culm-sheaths persistent; 10–15 cm long; 0.33 length of internode; purple; smooth; pubescent; hairy on margins; truncate at apex; auriculate; ciliate on shoulders. Culm-sheath ligule ciliate. Culm-sheath blade linear; acuminate. Leaf-sheaths smooth. Leaf-sheath oral hairs ciliate; deciduous. Leaf-sheath auricles falcate.

28. *Phyllostachys aurea* Carrie. ex A. & C. Riviere

Perennial; caespitose. Rhizomes elongated; leptomorph. Culms erect; 200–800 cm long; 20–30 mm diam.; woody. Culm-internodes similar in length, or abbreviated and closely packed at the base; semiterete; hollow; 8–10 cm long; yellow; smooth; distally glabrous. Culm-nodes swollen. Culm-sheaths deciduous; 12–18 cm long; yellow, or green; pubescent; hairy at the base; with white hairs; without auricles. Culm-sheath ligule 1–2 mm high; ciliate. Culm-sheath blade lanceolate; reflexed; 3–6 cm long. Leaf-sheaths glabrous on surface, or puberulous; outer margin hairy. Leaf-sheath auricles absent.
29. *Pseudosasa japonica* (Siebold & Zucc. ex Steud.) Makino ex Nakai


30. *Pseudostachyum polymorphum* Munro

Perennial; culms solitary. Rhizomes elongated; pachymorph. Culms erect, or leaning; 1500–2000 cm long; 30–35 mm diam.; woody. Culm-internodes terete; hollow; 20–23 cm long; dark green, or glaucous. Culm-sheaths pubescent; with appressed hairs; with dark brown hairs; truncate at apex; auriculate; ciliate on shoulders. Culm-sheath ligule dentate. Culm-sheath blade triangular; as wide as sheath at base; acuminate. Leaf-sheaths puberulous.

31. *Sasa palmata* Nebulosa


32. *Schizostachyum pergracile* (Munro) Majumdar

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1000–3000 cm long; 50–75 mm diam.; woody. Culm-internodes terete; hollow; 30–45 cm long; glaucous. Culm-sheaths 10–15 cm long; 0.9–1 times as long as wide; brown; hispid; with black hairs; auriculate; setose on shoulders; shoulders with curved hairs; shoulders with 7–12 mm long hairs. Culm-sheath blade ovate; cordate; 5 cm long; pubescent; acuminate.
Leaf-sheaths striately veined; glabrous on surface. Leaf-sheath oral hairs scanty; deciduous. Leaf-sheath auricles erect. Ligule an eciliate membrane.

33. *Thrysostachys oliveri* Gamble

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 1500–2500 cm long; 50–70 mm diam.; woody. Culm-internodes terete; 40–60 cm long. Culm-sheaths persistent; 0.75 length of internode; chartaceous; pubescent; hairy throughout; with white hairs; without auricles. Culm-sheath ligule 0.2 mm high. Culm-sheath blade linear; 20–25 cm long; pubescent.

34. *Thrysostachys siamensis* Gamble

Perennial; caespitose. Rhizomes short; pachymorph. Culms erect; 700–1300 cm long; 20–60 mm diam.; woody. Culm-internodes terete; with small lumen; 15–30 cm long. Culm-sheaths persistent; chartaceous; pubescent; hairy throughout; with white hairs; without auricles. Culm-sheath blade triangular. Leaf-sheath oral hairs lacking, or scanty.
Fig 3.1 Different species of bamboo

- *T. oliveri*
- *D. giganteus*
- *D. asper*
- *M. baccifera*
Fig 3.1 Different species of bamboo (CONTD....)

- **B. nutans**
- **B. balcooa**
- **D. strictus**
- **B. vulgaris**
Fig 3.1 Different species of bamboo (CONTD...)

- **B. pallida**
- **G. atroviolacea**
- **B. multiplex**
- **Sasa palmata**
Fig 3.1 Different species of bamboo (CONTD…)

*Bambusa wamin*  
*S. pergracile*

*Bambusa tulda*  
*P. aurea*
Fig 3.1 Different species of bamboo (CONTD...)

D. brandisii

D. membranaceous

D. hamiltonii

B. striata
Fig 3.1 Different species of bamboo (CONTD…)

- **B. polymorpha**
- **B. nana**
- **D. sikkimensis**
- **Guadua angustifolia**
Fig 3.1 Different species of bamboo (CONT'D...)

- O. scriptoria
- P. japonica
- T. siamensis
- B. bambos
3.3 Collection of leaf samples for extraction of genomic DNA

CTAB method of DNA isolation was followed for DNA extraction as per the protocol given in Manual of Molecular cloning (Sambrook and Russell, 2001). The following protocol was used for DNA extraction:

1. Fresh young healthy leaf samples were collected using aseptic scissors.
2. The leaf material was wrapped in aluminum foil, labeled and kept at 4°C.
3. After wiping with 70% ethyl alcohol, 200mg of leaf was weighed and ground to a fine powder in a precooled mortar and pestle using liquid nitrogen. Care was taken not to allow the samples to thaw.
4. The leaf powder was transferred to sterile microfuge tube containing 1ml extraction buffer (Table 3.1) prewarmed to 60°C with the help of a sterile spatula. The contents were mixed by gentle inversions and placed in a water bath at 60°C.

Table 3.2: Preparation of extraction buffer

<table>
<thead>
<tr>
<th>Stock (Appendix)</th>
<th>Volume taken</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris-Cl (pH-8.0)</td>
<td>10 ml</td>
<td>100 mM</td>
</tr>
<tr>
<td>0.5 M EDTA (pH-8.0)</td>
<td>4 ml</td>
<td>20 mM</td>
</tr>
<tr>
<td>5 M NaCl</td>
<td>28 ml</td>
<td>1.4 M</td>
</tr>
<tr>
<td>10% CTAB</td>
<td>20 ml</td>
<td>2 %</td>
</tr>
<tr>
<td>β-Mercaptoethanol</td>
<td>200 µl</td>
<td>0.2 %</td>
</tr>
<tr>
<td>20% SDS</td>
<td>0.75 ml</td>
<td>0.15 %</td>
</tr>
<tr>
<td></td>
<td>Total volume was made up to 100 ml using distilled water</td>
<td></td>
</tr>
</tbody>
</table>
5. The contents were gently mixed after every 10 minutes to keep the aqueous and organic phase emulsified.

6. After one hour the tube was removed from the water bath and allowed to cool to room temperature (R.T.).

7. Thereafter, 600 µl of 24:1 chloroform: isoamyl alcohol solution was added to the tube.

8. The contents were kept emulsified for 20 minutes on a rocker set at low speed (5 revolutions per minutes). The contents were centrifuged at ~19,500g (13,500 rpm, Hermle Table top centrifuge) at 20°C for 15 minutes.

9. The top aqueous phase was collected in a sterile 2ml microfuge tube using a 20 µl micropipette.

10. Equal volume of ice cold isopropanol was added to the aqueous phase. The contents were mixed gently by inversion to allow the DNA to precipitate.

11. The DNA strands obtained were then spooled out into a microfuge tube. To this 70% ethyl alcohol was added. This was allowed to stand for 10 minutes at R.T. The microfuge was centrifuged at 5000 rpm for 5 minutes (~9000 g, Hermle Table top centrifuge). The supernatant was decanted carefully. The 70% ethyl alcohol wash was repeated once more.

12. The pellet was air dried to remove the traces of alcohol.

13. The DNA was then dissolved in 200 µl of TE buffer (Appendix). Those samples that did not dissolve readily were kept at 60°C for 30 minutes to 1 h.

14. DNase free RNase was added to a final concentration of 10 µg/ml and the solution was incubated at 37°C for one hour.
15. For the removal of RNase, equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) (Appendix) was added. The two phases were mixed gently for 15 seconds and then centrifuged at 2500g for 2 minutes at R.T.

16. The upper aqueous phase was collected in a fresh microfuge tube. To it equal volume of Chloroform: Isoamyl alcohol (24:1) was added. The two phases were mixed gently for 15 seconds and then centrifuged at 2500g for 2 minutes at R.T.

17. The upper aqueous phase was collected in a microfuge tube. To it 1/10 volume of 3 M Ammonium acetate was added. It was gently mixed and equal volume of ice cold isopropanol was added. The content was mixed by inversion and was left for 30 minutes to 1 hour at -20°C for DNA precipitation.

18. The solution was centrifuged at ~19,500g (13,500 rpm, Rotor-220.87VO3/4, Hermle Table top centrifuge) for 15 minutes at 4°C. A 70% ethyl alcohol wash as described in step 11 was performed.

19. The pellet was then air dried and dissolved in 500 µl of TE buffer.

DNA extracted from the leaf material was then stored at -20°C and used as a template for downstream process.

3.4 Quantification of isolated genomic DNA through gel electrophoresis

The total DNA was quantified using Biophotometer (Eppendorf). The yield was determined by measuring the absorbance at A_{260} and A_{280}. The level of purity was determined by A_{260}/A_{280} ratio.
3.5 Determination of the quality of the isolated DNA

The quality of genomic DNA samples was assessed by running an aliquot on 0.7% agarose (Sigma-A9539) gels. The genomic DNA (1µl) was mixed with loading dye and added to the wells. A ladder was also run simultaneously. The samples were electrophoresed at 1-5 V/cm. The genomic DNA was visualized using a dye, ethidium bromide, which intercalates between the bases of DNA molecule and fluoresces when illuminated by UV rays. After visualization the DNA was diluted if required to 50 ng/µl concentration.

3.6 Preparation of Agarose gel

3.15 gm of agarose was mixed with 450 ml of 1X TAE buffer and boiled to obtain molten homogenous gel. To it 22.5 µl of ethidium bromide was added and mixed properly. The molten gel was then poured into the casting tray with 26 well comb. While pouring the gel care was taken to avoid air bubbles being introduced in the gel. Gel was allowed to set for 30 min. As the gel solidifies, 1X TAE was poured into it and the comb was gently removed. The tray was then placed into the tank and samples were then loaded.

3.7 Primers employed

The primers selected for the study were obtained from Bangalore GeNei and were as follows:

a) rbcL: The gene for large subunit of ribulose bisphosphate carboxylase/oxygenase (RUBISCO) enzyme located on the chloroplast genome is appropriate choice for inference of phylogenetic relationships at higher taxonomic levels. Because of its slow synonymous nucleotide substitution rates in comparison with nuclear genes and its
functional constraints that reduce the evolutionary rates of non-synonymous, \textit{rbcL} is considered to be more useful. The region \textit{rbcL} selected on the basis of its best characterized gene sequence.

\textit{b}) \textit{matK}: Among the chloroplast gene, \textit{matK} is one of the most rapidly evolving genes. It has length of about 1550 bp and encodes the enzyme maturase which is involved in splicing of Type II introns from RNA transcript. In all photosynthetic land plants so far examined, \textit{matK} is located within an intron of approximately 2600 bp positioned between the 5' and 3' exon of t-RNA gene for lysine, \textit{trnK}. It has the property of rapid evolution and ubiquitous presence in plants. PCR amplification was attempted using available primer set \textit{matK} X (F) and \textit{matK} 5 (R) in the present work. The \textit{matK} was selected for its easy amplification and alignment besides its desirable high interspecific but low intraspecific variation.

\textit{c}) \textit{trnH2-psbA}: This intergenic spacer is the most variable genome segment in the chloroplast of angiosperms. It has an average length of approximately 450 bp but varying from 296-1120 bp based on available data. The \textit{trnH2-psbA} locus has been successfully PCR amplified from a wide range of angiosperms and gymnosperms, ferns, mosses, and wild liverworts. However, problems were observed for obtaining high-quality bidirectional sequences and alignment of sequences in certain taxa due to high length variations. Nonetheless, a majority of the teams proposed the use of \textit{trnH2} and \textit{psbA} as a barcode for plants, mostly in combination with \textit{matK}. The CBOL Plant Working group found the species discriminatory power of \textit{trnH2} and \textit{psbA} to be the highest (69\%) among the seven loci tested and thus proposed it as the most preferred supplementary locus.
Therefore, *trnH2* and *psbA* can be used in a three locus barcode system wherever the two-locus barcode system fails to provide adequate resolution.

d) *rpoC1*: Genes *rpo B, rpoC1* and *rpoC2* encodes three out of the four subunits of the chloroplast RNA polymerase. Genome wide substitution analysis in family like *Geraniaceae* revealed that *rpoB, rpoC1 and rpoC2* accumulating higher amount of non-synonymous substitution, making these genes highly suitable for phylogenetic studies (Guisinger *et al.*, 2008). Primer set *rpoC1 2* (F) and *rpoC1 3* (R) were found to be best as in comparison to the other combinations.

Table No. 3.3: Primers for barcode and their PCR condition

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name</th>
<th>MW</th>
<th>Sequence</th>
<th>Amplified product Length</th>
<th>PCR Condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>rbcL1F</em></td>
<td>6076</td>
<td>ATGTGCACCACAAACAGAAAC</td>
<td>734bp</td>
<td>95/1min 95/30s 48/30s 68/1m 68/5m</td>
<td>Bafeel <em>et al.</em>, 2011</td>
</tr>
<tr>
<td></td>
<td><em>rbcL 724R</em></td>
<td>6096</td>
<td>TCGCATGTACCTGACGATGCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>matK X</td>
<td>6358</td>
<td>TAATTTACGATCAATTCATTC</td>
<td>734-821bp</td>
<td>94/1m 94/30s 53/40s 72/40s 72/5m</td>
<td>Kew Royal Botanic Gardens Phase 2 Update</td>
</tr>
<tr>
<td></td>
<td>matK 5</td>
<td>6154</td>
<td>GTTCTAGCACAAGAAAGTGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>rpoC1 2</em></td>
<td>6259</td>
<td>GGCAGAAGGGGAGATTTTC</td>
<td></td>
<td>94/1m 94/30s 53/40s 72/40s 72/5m</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>rpoC1 3</em></td>
<td>7143</td>
<td>TGAGAAAAACATAAGTAAAGCAGGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>trnH2</em></td>
<td>7028</td>
<td>CGCGCATGGTGGATTACAAATCC</td>
<td></td>
<td>94/3m 94/30s 55/30s 68/1m 68/10m</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>psbA</em></td>
<td>6403</td>
<td>CGAAGCTCCATCTACAAATGG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.8 PCR Amplification

The following protocol was adapted for amplification of the desired DNA fragment. A master mix was prepared in procedure because it allows the component to be mixed and divided uniformly for each reaction. The master mix included dNTP (0.2 mM each), Taq Buffer A (1X), Taq Polymerase (1U/reaction), forward primer and reverse primer (1µM each), template DNA (RBG Kew mat K protocol) and final volume was made to 20µl using sterile MB grade water for each reaction. The primers used in the study belong to plastid genes region *rbcL*, *mat K*, *rpoC1* and *trnH2-psbA*. The primers are described in the table 3.3.

3.9 PCR optimization

It is important to maintain consistency in the concentrations of the components of the PCR reaction to obtain good results. In addition to this appropriate PCR condition is also very important to be standardized. To curb this problem gradient PCR as well as different PCR conditions reported by several authors were analyzed to obtain intense, strong, unambiguous band.

  a) *rbcL*: For the *rbcL* region the primer sequence reported by Bafeel et al. (2011), were tested for their amplification. The PCR condition was same as mentioned in the article.

  b) *mat K*: Four different primer *matK* 5, *matK*2.1, *matK* 2.1a, *matK* X, were tested for their amplification in different combinations and the best one was chosen for further study.

  c) *rpoC1*: Different combinations of the four primer available such as *rpoC1* 1, *rpoC1* 2, *rpoC1* 3 and *rpoC1* 4 were tested for their amplification.

  d) *trnH2* and *psbA*: The primer pair were tested for their amplification at different reported PCR conditions.
PCR condition for amplification followed was accordingly as mentioned in Table 3. Sharp intense monomorphic unambiguous bands were considered for sequencing.

3.10 Agarose gel electrophoresis of amplicons

The product obtained after PCR was analyzed by agarose gel electrophoresis. For the analysis of the amplified fragment agarose (HIMEDIA) was used. 1% (m/v) agarose gels was prepared with 1% (m/v) agarose (HIMEDIA) and 1x TAE buffer [50x TAE (48.44 g Tris; 11.42 ml acetic acid; 2.92 g EDTA)]. After adding ethidium bromide into the molten gel, it was casted in the casting unit by placing the suitable comb. Care was taken to avoid air bubbles. The PCR product mixed with loading dye was loaded into the wells. Appropriate ladders were also run with the samples. The samples were electrophoresed at constant voltage of 150 Volts (5V/cm) for 3 hours.

The gel was then visualized under UV light on a transilluminator (Banglore GeNei) and documentation of the samples was done on G:Box Syngene system.

3.11 Elution of amplified DNA fragments for sequencing

These amplicons were eluted from 1% agarose gel (Himedia MB). The protocol was adapted from the directions indicated in the kit’s user manual. The eluted amplicons were purified using Qiagen Minelute® Gel extraction kit. The concentration of the eluted fragments was determined using Biophotometer Plus (Eppendorf).

1. The DNA fragment was excised from the agarose gel with a clean, sharp scalpel.
2. The gel slice was weighted in a colorless tube. 3 volumes of Buffer QG was added to 1 volume of gel (100 mg gel ~ 100 μl). The maximum amount of gel slice per spin column is 400 mg. For >2% agarose gels, add 6 volumes Buffer QG.

3. It was incubated at 50°C for 10 min (or until the gel slice has completely dissolved). The tube was vortexed every 2–3 min during incubation to help dissolve the gel.

4. After the gel slice had dissolved completely, the color of the mixture was checked to be yellow similar to Buffer QG without dissolved agarose. If the color of the mixture was orange or violet, 10 μl of 3 M sodium acetate, pH 5.0, was added and mixed. The color of the mixture then turned to yellow.

5. 1 gel volume of isopropanol was added to the sample and mixed by inverting.

6. MinElute spin column was placed in provided 2 ml collection tube.

7. Sample was applied to the MinElute column and centrifuged for 1 min. until the entire sample has passed through the column. Flow-through was discarded and the MinElute column was placed back into the same collection tube. For sample volumes of more than 800 μl, the process was repeated in the same tube.

8. 500 μl Buffer QG was added to the MinElute column and centrifuged for 1 min. Flow-through was discarded and the MinElute column was placed back into the same collection tube.

9. 750 μl Buffer PE was added to MinElute column and centrifuged for 1 min. Flow-through and was discarded and the MinElute column was placed back into the same collection tube.

10. The column was centrifuged in a 2 ml collection tube (provided) for 1 min. Flow-through was discarded before this additional centrifugation in order to completely remove the residual ethanol from Buffer PE.
11. Each MinElute column was placed into a clean 1.5 ml microcentrifuge tube. To elute DNA, 10 μl Buffer EB (10 mM Tris·Cl, pH 8.5) or water was added to the center of the MinElute membrane. The column was allowed to stand for 1 min. and then centrifuged for 1 min.
12. The purified DNA was analyzed on a gel, by mixing 1 volume of Loading Dye to 5 volumes of purified DNA.

Note: All centrifugation steps were carried out at 17,900 x g (13,000 rpm) in a table-top microcentrifuge (Hermle).

3.12 Sequencing

All the DNA regions were sequenced by using the BigDye® Terminator v3.1 Cycle Sequencing Kit according to the protocol provided. The sequencing of the amplicons included three steps:

1. For cycle sequencing:

   To prepare reaction mix-

   a. For each reaction the following reagents are to be added into a separate tube (Table 3.3).

Table 3.4 Preparation of reaction mixture

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Terminator ready reaction mix</td>
<td>8.0 μl</td>
</tr>
<tr>
<td>2</td>
<td>Amplicon</td>
<td>5-20 ng</td>
</tr>
<tr>
<td>3</td>
<td>Primer</td>
<td>3.2 p.mol</td>
</tr>
<tr>
<td>4</td>
<td>Deionised water</td>
<td>q.s.</td>
</tr>
<tr>
<td>5</td>
<td>Total volume</td>
<td>20μl</td>
</tr>
</tbody>
</table>
b. Reagents were mixed well and briefly spun

Cycle sequencing was done on 9700 thermal cycler GeneAmp® PCR System 9700. To sequence the amplicons on the following conditions were undertaken.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The tubes were placed in the thermal cycler and volume was correctly set to 20 µl</td>
</tr>
</tbody>
</table>
| 2.   | Initial denaturation was done  
|      | a. Rapid thermal ramp to 96°C  
|      | b. 96°C for 1 min. |
| 3.   | Repeat the following for 25 cycles  
|      | • Rapid thermal ramp to 96°C  
|      | • 96°C for 10 sec  
|      | • Rapid thermal ramp to the respective annealing temperature  
|      | • Annealing temperature for 5 sec  
|      | • Rapid thermal ramp to 60 °C  
|      | • 60 °C for 4 min. |
| 4.   | Rapid thermal ramp to 4 °C and hold until ready to purify |
| 5.   | Tubes were shortly spun |
| 6.   | Purification steps were undertaken |

2. Purification by Ethanol/EDTA/Sodium Acetate Precipitation:

To precipitate 20- µL sequencing reactions in 96-well reaction plates:

a. The 96-well reaction plate was removed from the thermal cycler and briefly spun.

b. 2 µL of 125 mM EDTA was added to each well.

c. 2 µL of 3 M sodium acetate was added to each well.
d. 50 μL of 100% ethanol was added to each well.

e. The plate was sealed with aluminum tape and mixed by inverting 4 times.

f. Incubation was done at room temperature for 15 min.

g. Plate adapter was used and the plate was spun at the maximum speed at 1400–2000 × g for 45 min or 2000–3000 × g for 30 min

h. The plate was inverted and spun up to 185 × g, then removed from the centrifuge.

i. 70 μL of 70% ethanol was added to each well.

j. With the centrifuge set to 4 °C, spinning was done at 1650 × g for 15 min.

k. The plate was inverted and spinning was done up to 185 × g for 1 min, then removed from the centrifuge.

l. To continue, samples were resuspended in injection buffer. To store, cover with aluminum foil, and store at 4 °C.

3. Performing sample electrophoresis:

The samples were resuspended in 11 μl Hi di formamide and subjected to electrophoresis.

3.13 Analysis of the sequences obtained using MEGA 5.2.2 software MEGA

Molecular Evolutionary Genetics Analysis, is freely available software for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees. The programme was used for alignment of the sequences obtained from each primer among 34 species of bamboo studied using ‘MUSCLE’. The aligned sequences were tested for the ‘Best substitution model for further generation of phylogenetic tree.
RESULTS

4.1 Quantification and assessment of quality of isolated genomic DNA through gel electrophoresis

The quality and quantity of genomic DNA of all 34 species of Bamboo was assessed by 0.7% agarose gel electrophoresis. Though some degree of shearing was observed, the DNA was largely high molecular weight and suitable for the present study. The yield of DNA was also found to be fairly uniform ranging from 85.2 in *Cephalostachyum capitatum* to 1570.8 in *Dendrocalamus hamiltonii*. The DNA yield in 34 target species was found suitable for further molecular work. The concentration of DNA was determined using Biophotometer plus (Eppendorf) (Table 4.1).

Table 4.1 Concentration of DNA of 34 species of bamboo

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the species</th>
<th>Concentration (in ng/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bambusa balcooa</em></td>
<td>657.2</td>
</tr>
<tr>
<td>2</td>
<td><em>Bambusa bambos</em></td>
<td>517.7</td>
</tr>
<tr>
<td>3</td>
<td><em>Bambusa multiplex</em></td>
<td>673.1</td>
</tr>
<tr>
<td>4</td>
<td><em>Bambusa nana</em></td>
<td>106.3</td>
</tr>
<tr>
<td>5</td>
<td><em>Bambusa nutans</em></td>
<td>343.8</td>
</tr>
<tr>
<td>6</td>
<td><em>Bambusa pallida</em></td>
<td>295.3</td>
</tr>
<tr>
<td>7</td>
<td><em>Bambusa polymorpha</em></td>
<td>195.2</td>
</tr>
<tr>
<td>8</td>
<td><em>Bambusa striata</em></td>
<td>479.5</td>
</tr>
<tr>
<td>9</td>
<td><em>Bambusa tulda</em></td>
<td>375.3</td>
</tr>
<tr>
<td>10</td>
<td><em>Bambusa vulgaris</em></td>
<td>104.3</td>
</tr>
<tr>
<td>11</td>
<td><em>Bambusa wamin</em></td>
<td>516.4</td>
</tr>
<tr>
<td>12</td>
<td><em>Cephalostachyum capitatum</em></td>
<td>85.2</td>
</tr>
<tr>
<td>13</td>
<td><em>Dendrocalamus asper</em></td>
<td>709.7</td>
</tr>
<tr>
<td>14</td>
<td><em>Dendrocalamus brandisii</em></td>
<td>741.0</td>
</tr>
<tr>
<td>15</td>
<td><em>Dendrocalamus giganteus</em></td>
<td>381.4</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Name of the species</td>
<td>Concentration (in ng/µl)</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>16</td>
<td><em>Dendrocalamus hamiltonii</em></td>
<td>1570.8</td>
</tr>
<tr>
<td>17</td>
<td><em>Dendrocalamus membranaceus</em></td>
<td>590.9</td>
</tr>
<tr>
<td>18</td>
<td><em>Dendrocalamus sikkimensis</em></td>
<td>88.8</td>
</tr>
<tr>
<td>19</td>
<td><em>Dendrocalamus strictus</em></td>
<td>649.8</td>
</tr>
<tr>
<td>20</td>
<td><em>Dinochloa andamanica</em></td>
<td>92.2</td>
</tr>
<tr>
<td>21</td>
<td><em>Dinochloa MacClellandii</em></td>
<td>1272.6</td>
</tr>
<tr>
<td>22</td>
<td><em>Gigantochloa atroviolacea</em></td>
<td>401.9</td>
</tr>
<tr>
<td>23</td>
<td><em>Gigantochloa macrostachya</em></td>
<td>518.5</td>
</tr>
<tr>
<td>24</td>
<td><em>Gigantochloa nigrociiliata</em></td>
<td>290.6</td>
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<tr>
<td>25</td>
<td><em>Guadua angustifolia</em></td>
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<tr>
<td>26</td>
<td><em>Melocanna baccifera</em></td>
<td>236.7</td>
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<tr>
<td>27</td>
<td><em>Ochlandra scriptoria</em></td>
<td>326.8</td>
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<tr>
<td>28</td>
<td><em>Phyllostachys aurea</em></td>
<td>232.9</td>
</tr>
<tr>
<td>29</td>
<td><em>Pseudosasa japonica</em></td>
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<tr>
<td>30</td>
<td><em>Pseudostachyum polymorphum</em></td>
<td>106.3</td>
</tr>
<tr>
<td>31</td>
<td><em>Sasa palmata</em></td>
<td>473.3</td>
</tr>
<tr>
<td>32</td>
<td><em>Schizostachyum pergracile</em></td>
<td>600.0</td>
</tr>
<tr>
<td>33</td>
<td><em>Thrysostachys oliveri</em></td>
<td>222.1</td>
</tr>
<tr>
<td>34</td>
<td><em>Thrysostachys siamensis</em></td>
<td>309.0</td>
</tr>
</tbody>
</table>

The DNA was diluted for use in further amplification studies. The total genomic DNA extracted from 200mg leaf material had been resuspended in 500 µl T.E. and 2 µl had been used to perform electrophoresis.

### 4.2 Standardization of PCR conditions

Four primer pairs were tested for their amplification in 34 species of bamboo used.

1. *rbcL* 1F and 724R: The reported PCR condition gave sharp, intense, unambiguous band of 738 bp which were monomorphic in nature and present in all studied species of bamboo (Plate 4.2.). Thus, no standardization of PCR condition was required for this primer pair.
2. *matK* X and 5: Different forward and reverse primers for the *matK* region were available, such as *matK*390, *matK* X, *matK* 2.1, *matK*2.1a as forward primer and *matK*1326, *matK* 5 as reverse primer, which were tested for unambiguous and sharp amplification. The primer combination *matK* X as forward and *matK* 5 as reverse yielded sharp, single, unambiguous and monomorphic band of 930 bp in all 34 species of bamboo (Plate 4.3).

3. *trnH2* and *psbA*: Gradient PCR was performed to standardize the annealing temperature and best amplification was obtained at 55°C. The extension temperature was also required to be decreased from 72°C to 68°C for amplification. *trnH2-psbA* primer pair resulted into 88.23% PCR success rate which corresponds to positive amplification in only 30 species. Amplicons considered for further sequencing was of 635 bp molecular weight (Plate 4.4).

4. *rpoC1*2 and 3: Two forward and two reverse primers were tested for amplification in four combinations. Primer pair yielding single, sharp and intense band was considered which resulted into amplicon of 450bp in all 34 species of bamboo (Plate 4.5).

**4.3 Sequencing of amplicons**

1. The amplicons thus obtained were subjected to sequencing. 100% sequencing were attained for the primer set *rbcL1F*-724R, *matK* X-5 and *rpoC1* 2-3. The primer set *trnH2-psbA* showed no amplification in *Bambusa multiplex*, *Dendrocalamus sikkimensis*, *Dendrocalamus strictus* and *Schizostachyum pergracile* resulting into sequencing of rest of the amplicons of 30 species.
Plate 4.1a Gel picture of 24 species of Bamboo, M-λ HIND III

1 B. balcooa 7 B. polymorpha 13 D. asper 19 D. strictus
2 B. bambos 8 B. striata 14 D. brandisii 20 D. andamanica
3 B. multiplex 9 B. tulda 15 D. giganteus 21 D. macclellandii
4 B. nana 10 B. vulgaris 16 D. hamiltonii 22 G. atroviolacea
5 B. nutans 11 B. wamin 17 D. membranaceus 23 G. macrostachya
6 B. pallida 12 C. capitatum 18 D. sikkimensis 24 G. nigrociliata

Plate 4.1b Gel picture of 10 species of Bamboo, M-λ HIND III

25 G. angustifolia 31 S. palmata
26 M. baccifera 32 S. pergracile
27 O. scriptoria 33 T. oliveri
28 P. aurea 34 T. siamensis
29 P. japonica
30 P. polymorphum
Plate: 4.2. Amplicons obtained with \textit{rbcL}1F and 724R. M-100 bp molecular ladder

Plate 4.3. Amplicons with \textit{matK} X and \textit{matK} 5, M-100 bp molecular ladder.
Plate 4.4 Amplicons obtained with trnH2 and psbA, M- Low range DNA ruler

Plate 4.5 Amplicons obtained with rpoC1 2 and 3, M- Low range DNA ruler
4.4 Subjection of the sequences to MEGA 5.2.2 software

Once the sequences have been obtained they were subjected to MEGA 5.2.2 software. Molecular Evolutionary Genetics Analysis version 5 (MEGA5), enables mining online databases, building sequence alignments and phylogenetic trees, and using methods of evolutionary bioinformatics in basic biology, biomedicine, and evolution.

4.4.1 Sequence homology

An input file in the form of FASTA format was prepared and subjected for multiple sequence alignment using MUSCLE (MUltiple Sequence Comparison by Log- Expectation) (Fig 4.1-4.5). All the 34 sequences obtained from each primer were aligned. Once alignment was successfully completed, the primer pairs rbcL yielded sequences ranging from 584 bp for B.nana to 653bp for T.oliveri; matK ranging from 624-772 bp (G. angustifolia to B. striata); trnH2-psbA ranging from 505-524 bp (M. baccifera to D. maclellandii) and rpoC1 2-3 ranging from 398-403 bp (B.balcooa, B multiplex, G. atroviolacea to B. striata). Those sequences which were aligned were indicated with a asterick (*) sign at the first row and the column containing even a single different base was blank. The four base were indicated with four different coloured boxes such as A in green, G in purple, T in red and C in blue.
Fig 4.1 Sequences obtained from *rbcL* primer set were aligned using MUSCLE
Fig 4.2. Sequences obtained from *matK* primer set were aligned using MUSCLE
Fig 4.3. Sequences obtained from *trnH2* and *psbA* primer set were aligned using MUSCLE (34 samples)
Fig 4.4. Sequences obtained from *trnH2* and *psbA* primer set were aligned using MUSCLE (30 samples)
Fig 4.5. Sequences obtained from *rpoC1* 2 and 3 primer set were aligned using MUSCLE
Alignment of the sequences was further proceeded by analyzing for the ‘Best Substitution Model’. It determines the best criteria appropriate for generation of phylogenetic tree. According to the software, models with the lowest BIC scores (Bayesian Information Criterion) are considered to describe the substitution pattern the best. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (lnL), and the number of parameters (including branch lengths) are also determined (Fig 4.6-4.9).

Twenty four different nucleotide substitution models were tested to obtain the lowest BIC and AIC value. The primer pair rbcL had lowest BIC value 2649.449 and lowest AIC value 2130.355, matK had lowest BIC value 4176.833 and lowest AIC value 3677.898, trnH2-psbA had lowest BIC value 1021.958 and lowest AIC value 673.067 and rpoC1 had lowest BIC value 2442.442 and lowest AIC value 1940.272 respectively. On the basis of these lowest BIC and AIC value best model was selected which was utilized to generate phylogenetic tree.
Fig 4.6. Best substitution model for rbcL
Fig 4.7. Best substitution model for matK
Fig 4.8. Best substitution model for *trnH2* and *psbA*
Fig 4.9. Best substitution model for \textit{rpoC1} 2 and 3
Table 4.2 Summarization of primer-wise data

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Primer Name</th>
<th>Length of Amplicons (in bp)</th>
<th>PCR success rate</th>
<th>Best substitution model after trimming ambiguous ends</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rbcL</td>
<td>738</td>
<td>100%</td>
<td>JC+G</td>
</tr>
<tr>
<td>2</td>
<td>matK</td>
<td>930</td>
<td>100%</td>
<td>K2</td>
</tr>
<tr>
<td>3</td>
<td>trnH2 and psbA</td>
<td>635</td>
<td>88.23%</td>
<td>T92+G</td>
</tr>
<tr>
<td>4</td>
<td>rpoC1 2&amp;3</td>
<td>450</td>
<td>100%</td>
<td>K2+I</td>
</tr>
</tbody>
</table>

On the basis of lowest BIC scores (Bayesian Information Criterion), best substitution model selected for *rbcL* was Jukes-Cantor+ Gamma (rates among sites), *matK* was Kimura 2-parameter, *trnH2-psbA* was Tamura 3-parameter+gamma and *rpoC1 2-3* was Kimura 2-parameter + evolutionarily invariable respectively (Table 4.2). These selected models with their rates were utilized for generation of dendrogram.

4.4.2 Generation of phylogenetic tree

The phylogenetic tree represents the estimates historical relationship among the taxa and their ancestors. Four dendrograms were generated for each of the primer pair. They are given below:

1. In the dendrogram generated from *rbcL* primer pair (Fig 4.10), 34 number of species were divided into six divisions. The members belonging to subtribe III namely *Cephalostachyum capitatum, Dendrocalamus asper, Dendrocalamus brandisii, Dendrocalamus membranaceus, P. polymorphum* were grouped together along with the members of subtribe II such as *B. balcooa, B. nana, G. atroviolacea*. One member of subtribeIV (*D. macclellandii*) was also present in the same division. In the second division *S.palmata and P. japonica* belonging to subtribe I were present along with
other members of subtribe I namely *B. wamin, B. multiplex, B. striata, B. vulgaris.*

Mixed kind of distribution of different species of bamboo was observed at this locus.

2. Dendrogram generated from *matK* showed that the two species belonging to subtribe I (*O. scriptoria* and *M. baccifera*) occupies their position under same division. *B. nutans, B. striata, B. vulgaris* were nearby to one another occupying same subdivision. The boot strap values for different nodes were found to be highest as compared to other dendrograms therefore the reliability of this locus is greater as compared to the other studied loci. According to the *matK* dendrogram *T. oliveri, B. balcooa, D. asper* and *G. angustifolia* stand separately from the other species (Fig 4.11). Dendrogram shows greater number of branches which shows that the bamboo species considered are genotypically different from each other and does not fall into one group. The members belonging to on genera are also located under different divisions. Closely similar *B. tulda* and *B. nutans* were present under different subdivisions. While *B. striata* and *B. vulgaris* were present under different subdivision.

3. The dendrogram generated from the primer pair *trnH2-psbA* showed the greatest number branches among the four primer pair studied (Fig 4.12). Eighteen of the 34 species of bamboo occupied separate division which shows that these all species are independent from each other. The dendrogram was divided into two divisions with one division containing *B. polymorpha, B. balcooa and O. scriptoria*. The other division contained members of *G. macrostachya, G. nigrociliata* and *G. atroviolacea* positioned nearby followed by 5 members of *Dendrocalamus* genera such as *D. asper, D. brandisii, D. giganteus, D. hamiltonii* and *D. membranaceus* clumped
together. A clear cut division is exhibited genera-wise, resulting into the analysis of phylogenetic evolution. The primer sets proves to be efficient in phylogenetic analysis.

4. The dendrogram generated from *rpoC1* primer pair also yielded greater number of branches. Closely similar *B.tulda* and *B. nutans* species were present under different division (Fig 4.13). Dendrogram generated from rpoC1 showed a common ancestry of *B. nana*, *B. multiplex* and *B. vulgaris*. The two species *B. nutans* and *B. striata*; and *B.tulda* and *B. balcooa* occupied very close position to each other, exhibiting their common ancestor respectively. The overall dendrogram generated showed a mixed distribution of different species considered, thus exhibiting low resolvability of phylogenetic background.

5. The sequence data generated from four primer pair were concatenated using the software “SEAVIEW” and a combined dendrogram has been generated representing all the sequences (Fig 4.14). The four species *T. oliveri*, *B. balcooa*, *D. asper* and *G.angustifolia* occupied a separate position in the dendrogram.
Fig 4.10 Dendrogram generated by *rbcL*
Fig 4.11. Dendrogram generated by *matK* X-5
Fig 4.12. Dendrogram generated by \textit{trnH2-psbA}
Fig 4.13 Dendrogram generated by rpoC1 2-3
Fig 4.14. Dendrogram generated by concatenating the sequences obtained from four primers
4.5 Estimates of Average Evolutionary Divergence over all Sequence Pairs

Estimation of mean diversity in entire population was computed which resulted into overall mean distance of 0.006 for \textit{rbcL}, 0.049 for \textit{matK}, 0.016 for \textit{trnH2-psbA} and 0.012 for \textit{rpoC1}. Standard error estimate(s) are shown in the second column and were obtained by a bootstrap procedure (100 replicates) (Table 4.3).

Table 4.3 Overall genetic diversity existing in entire population

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Name of locus</th>
<th>Overall mean distance</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\textit{rbcL}</td>
<td>0.006</td>
<td>0.002</td>
</tr>
<tr>
<td>2</td>
<td>\textit{matK}</td>
<td>0.049</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>\textit{trnH2-psbA}</td>
<td>0.016</td>
<td>0.003</td>
</tr>
<tr>
<td>4</td>
<td>\textit{rpoC1}</td>
<td>0.012</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Submission of the sequences to the NCBI database

Sequences were submitted to NCBI through ‘BANKIT’ in order to obtain the accession number.

List of some of the accession numbers with their sequences are given below (Table 4.4):

Table 4.4 List of accession numbers

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Name of species</th>
<th>Gene</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\textit{Bambusa balcooa}</td>
<td>ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (\textit{rbcL}) gene</td>
<td>KU558924</td>
</tr>
<tr>
<td>2</td>
<td>\textit{Bambusa multiplex}</td>
<td>KU647273</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>\textit{Bambusa nana}</td>
<td>KU647274</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>\textit{Bambusa nutans}</td>
<td>KU647275</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>\textit{Bambusa pallida}</td>
<td>KU647276</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>\textit{Bambusa polymorpha}</td>
<td>KU647277</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>\textit{Bambusa striata}</td>
<td>KU647278</td>
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</tbody>
</table>
Sequences of some bamboo species obtained from the primer set \textit{rbcL}

\textbf{>Seq1 [organism= \textit{Bambusa balcooa}]}
CAATGGCCATTGCCGAGTAACTCCTCAGCCGGGGTCCGCCCGAAGAAGCAGGGGCTGCAGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTTTGGACTGATGGACCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATTTTGTTTCAAGCGCCCTACG

\textbf{>Seq3 [organism= \textit{Bambusa multiplex}]}
AAACCAAGGATAACTCGATATCTTGGCAGCATCGCTCCGAGATTCCTCAGCCGGGGTTCCGCCCGAAGAAGCAGGGGCTGCAGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTTTGGACTGATGGACCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATTTTGTTTCAAGCGCCCTACG

\textbf{>Seq4 [organism= \textit{Bambusa nana}]}
TGGTGTGCGTTTCATTAGTGCCCGGATCTTCTACTTCTGGAATACGCTCCGAGATTCCTCAGCCGGGGTTCCGCCCGAAGAAGCAGGGGCTGCAGTAGCTGCCGAATCTTCTACTGGTACATGGACAACTGTTTGGACTGATGGACCTTACCAGTCTTGATCGTTACAAAGGACGATGCTATTTTGTTTCAAGCGCCCTACG

110
>Seq5 [organism= Bambusa nutans]
AAACCAAGGATACTGATATCCTTGCAGCAGTACCTCCGAGTAACTCCTCAACGCGGGGGGTTC
CGCCCGAAGAACAGAAAGGCTGGTACTGTGCAGATATCTGGGACACGTCTTCTCAAGCTCA
ACTGTTTGGACTGATGGACTTGGTAAGGTACGCTTCTACCTACTCTTATCCTATTCAAGCT
ATCGAGGCTGTTGTGGAGAGGAAAATCAATATATCGTATTAGTACTTTATTTAATCC
GACCTATTGAGAGGTTCTCTTTACTAAACTACAACCCCGTTGGAATCCCGGGCAG
TTTGGTCTCTACACCCTTGCTCTACGTCTGGAGGATCTGCGAATTCCCATTTATTCA
TTTGGTCTCTAACAGCATACGCAAGTAAACATGCTATTACCCACATCTAA
>Seq6 [organism= Bambusa pallida]
AAACCAAGGATACTGATATCCTTGCAGCAGTACCTCCGAGTAACTCCTCAACGCGGGGGGTTC
CGCCCGAAGAACAGAAAGGCTGGTACTGTGCAGATATCTGGGACACGTCTTCTCAAGCTCA
ACTGTTTGGACTGATGGACTTGGTAAGGTACGCTTCTACCTACTCTTATCCTATTCAAGCT
ATCGAGGCTGTTGTGGAGAGGAAAATCAATATATCGTATTAGTACTTTATTTAATCC
GACCTATTGAGAGGTTCTCTTTACTAAACTACAACCCCGTTGGAATCCCGGGCAG
TTTGGTCTCTACACCCTTGCTCTACGTCTGGAGGATCTGCGAATTCCCATTTATTCA
TTTGGTCTCTAACAGCATACGCAAGTAAACATGCTATTACCCACATCTAA
>Seq7 [organism= Bambusa polymorpha]
TTTGGCCCGGGCGAGATGAAGACAGCGGGTCTGCAGTAGTGGCAGATCCTTTCTACTGTTAC
ATGGACACCTGTGGATGGCTTACAGATCTTTGTTAGCTAAAAAGGCGACGT
CTATCACATCGAGGCCCCTGGTTGGGAGGAAAATCAATTATATCGTATTAGTACTTT
TCCATTAGACCTATTGGAAGGAGGGTTCTCTTTACTAACAAGCTTTTATCCCATTTG
AACTGATTATTGTTTTCAAAGCCTACGCGCCTCTACGCTCTGGGAGGATCTGCAGATTCC
CCTACTTATTCAAAAACCTTTTCCAAGGCTGCTACGCTACGTCGCCGACTTTATCAGGAT
AAGTTGAAGATAGTGAGGGGGTCCTTTTTTGGGAGTACTATTAAACAAAAATGGGA
TTACCCGAAAAAATTACCGTGAAGCGTGTTATAGTGATCTACGCGGCTGAGACTTTG
TTTACAAAGATCGATGAAAACGTAAAACCATTTCTGCTGAGGGGGCGCTGGTTTTTGC
TTTTGTCCTTTTGCCGGAAGCATAATTATATACAGACGGCGAATCAGGAGG
>Seq8 [organism= *Bambusa striata*]

AAACCAAGGATACTGCTATCTTGGCAAGCATTCTCCGAGTAACTCCTCAGCCGGGGGTTTC
CGCCCAAGAAGCAGGGGGCTGCGAGTAGTGGCAGCTCCTACTTACTGGTACATGGACA
ACTGTATTGGACTGATGGAATTACTTTACAGTGTTGATCGTTACCAAAAGGACGATGCTATCAC
ATCGAGCCCGTTTGGGAGGAGAAAAATCAATATATCAGGTTATGATAGCATTATCCATTA
GACCTATTTGAAGAGGTTTCTGTATTACCAACATGTTTACTTTACATGGGGTAACGTAT
TTGGTTTCAAGCCCTACGCGCTCTACGTCTGGAGGAGACTGCAATTCCCCCTACTTTA
TTCAAAAAACTTTCCATTAGGTCGGCCTCATGGATTCAAGTTGAAAGGGATAAGTTGAA
CAAGTACGGCCGTCTTTTTTGGGATGACTATTTAAAACAAAAATTGGGATTTATCCGC
AAAAAATTATGGTAGAGCGTGTTAGTAGTGGTCTACCGGGTGGACTTTACCCAAAA
AGATGATGAAACGTAACACTCAACACCATTATGCGCTGGAGGGACCGTTTTGTCTTT
TTTGCCAGAAGCATTATTTATAAGCAGCAGCCGAAACCGGTGAAATCAAGGGGGCAT
TACTTTGAATGCTACTGCAGGA