Chapter 16
Molecular Markers in Bamboo Genotyping: Prospects for Conservation and Breeding

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Abstract Bamboo (family: Poaceae) is a cash crop, and due to its economic benefit, this gift of nature is considered as "green gold." This widely distributed plant group is facing concern regarding its conservation due to its continually increasing demand. The successful conservation of plant species lies in proper identification and characterization. In the case of bamboo, as the flowering cycle is long, the identification and taxonomical classification is dependent on mainly its vegetative features like culm and culm-sheath characters. Due to its inappropriate flowering cycle as well as widespread polyploidization of the genome, the taxonomy of bamboo is highly unstable. The molecular techniques in taxonomic classification have been employed since its discovery as it is not influenced by external factors. Molecular taxonomy can resolve many discrepancies regarding the classification and identification of genotypes which are long-standing and could not be solved based on phenotypic characters. Molecular descriptors such as hybridization-based marker like restriction fragment length polymorphism (RFLP) or polymerase chain reaction (PCR)-based markers like random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), simple sequence repeat (SSR), sequence characterized amplified regions (SCARs), and amplified fragment length polymorphism (AFLP) or sequence-based marker like single-nucleotide polymorphism (SNP), diversity array technology (DArT), etc. are used to evaluate the genetic diversity as well as for accurate identification of the bamboo species. The present study will elaborate on the utility of different molecular markers for identification and taxonomic classification in bamboos.

Keywords Bamboo · Molecular breeding · Molecular markers · Molecular taxonomy · Molecular phylogeny

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16.1 Introduction

Bamboo, an economically important plant species, belongs to the grass family (Poaceae). This diverse plant group constitutes a single subfamily Bambusoideae distributed under 121 genera and 1662 species (Canavan et al. 2017). Subfamily Bambusoideae is further grouped into three major tribes, viz., temperate woody bamboos consisting of 546 species, tropical woody bamboos having 812 species, and herbaceous bamboos consisting of 124 species (Clark et al. 2015). It is distributed globally except Europe which has no native species (Liese and Hamburg 1987); the Asia-Pacific and South America have the maximum species diversity, whereas Africa has the minimum bamboo species diversity (Bystriakova et al. 2003). The highest bamboo-producing country in the world is China which produces 164 million tons of bamboo yearly with a productivity of 30 tons/ha (Lobovikov et al. 2007). Due to its multifarious utility and versatile benefit in day-to-day life of human beings, it is named as "green gold" or in someplace "poor man's timber." It has cultural (Kurz 1876), artistic (McNeely 1995), religious (Skeat 1900), as well as economic value. According to the 2015–2016 National Institution for Transforming India (NITI Ayog) report, 136 million bamboo cultivators in India fetch approximately 1750 USD annual income from bamboo (NITI Policy Paper 2017). Bamboo plays a manifold role in environmental protection such as preventing soil erosion and conserving soil moisture (Christanty et al. 1996, 1997; Mailly et al. 1997). It can produce 30% more oxygen than an equivalent stand of other trees (Prieto et al. 2013). It provides food for many wild animals and thus plays a great role in the forest ecosystem. Research has proven that bamboo is nine times stronger than that of commercial geocell material and thus can be useful in soft ground engineering (Hegde and Sitharam 2014). Many parts of bamboo plants have therapeutic values also, and it has manifold use in Ayurvedic medicine (Das et al. 2012; Nirmala et al. 2018; Ren et al. 2019).

Keeping aside the aforesaid importance of bamboo in human life, it is exploited irrationally for a long time. Due to irrational utilization or exploitation and genetic erosion of bamboo species, collection and preservation of germplasms is a necessary task now (Thomas et al. 1988; Loh et al. 2000; Nilkanta et al. 2017), along with the classification and identification of the species which becomes the need of the hour (Rao and Rao 1995; Bahadur 1979; Soderstrom and Calderon 1979). To protect the species, conservation and utilization of the balance of germplasms, characterization, and identification are a special need (Nayak et al. 2003; Liu et al. 2013; Mei et al. 2014; Desai et al. 2015; Migicovsky et al. 2019). During the recent past, good numbers of articles have been published discriminately using different methods of molecular genotyping in bamboo. In the present article, the aim is to summarize all the information related to molecular genotyping as evident from the published literatures, which ultimately will help in breeding and conservation of the bamboo species across the globe.

16.2 Morphometric Taxonomy in Bamboo

Traditionally classification and identification of bamboo are based on morphological characters. In modern times, technologies like biochemical, anatomical, and physiological features are also included for the identification of bamboo species (Stapleton 1997). The flowering cycle in the bamboo species ranged between 3 and 120 years (Janzen 1976). So, the morphology-based classification of bamboo is mainly dependent on nonreproductive characters like culm and culm-sheath characters. The vegetative features are often influenced by the environment (Wu 1962) and thus less trustworthy. As reported by Shalini et al. (2013), vegetative features that demarcate species may be more delicate and inaccessible for study which is also a great cause of concern regarding bamboo identification.

In the year 1896, Prof. Gamble identified most of the old world bamboos based on flowers and vegetative descriptors. Later on, many workers have suggested different morphological parameters such as culm-sheath characters (Chatterjee and Raizada 1963), young vegetative shoot, and branching pattern (Bennet et al. 1990). Later Bhattacharya et al. (2006) and Das et al. (2007) utilized 32 key morphological parameters for phylogenetics relationship study in bamboo species. The dendrogram generated using key morphological parameters was not in compliance with the species as reported by Gamble (1896). A gregarious flowering bamboo, *Thamnocalamus spathiflorus* subsp. *spathiflorus*, was studied by Bhattacharya et al. (2009). The vegetative and floral morphology illustrated was in conformity with the previous report (Naithani et al. 2003; Clayton et al. 2006). Attigala et al. (2016) studied *Kuruna*, a new temperate woody bamboo genus, and it included seven species distributed in Sri Lanka and South India. This study provides a reorganized report on the morphology of the genus *Bamboo*, a comprehensive description of seven bamboo species, detailed phylogenetics of entire species, and a morphological descriptor useful for their identification. This study includes *Arundinaria wightiana* in *Kuruna* based on its morphology and which is the only prevalent *Kuruna* species in India.

16.3 Limitations of Morphometric Taxonomy

There are several instances of taxonomical discrepancies regarding the classification of bamboo species based on vegetative characters. Soderstrom and Ellis (1987) have transferred one species of *Oxytenanthera* (*Oxytenanthera monadelpha*) to a new genus *Pseudoxytenanthera* based on its vegetative and floral characteristics, but later *Pseudoxytenanthera* was merged with *Oxytenanthera* by Majumdar (1989) due to its similarity with type specimen, *Oxytenanthera abyssinica*. As opined by Sharma (1996), morphological features are not consistent to distinguish the two genera, viz., *Pseudoxytenanthera* and *Oxytenanthera munro*, and as a result, the genus name *Oxytenanthera* persisted. Das et al. (2007) reported vegetative parameters alone are

incapable of distinguishing strongly associated species. The cluster pattern of 15 bamboo species was not in accordance with the classification given by Gamble (1896). In this context, a need for a different approach to classification and identification of bamboo was raised. In taxonomical classification, a new concept of integrative taxonomy appeared which is a multidimensional approach and considers multiple lines of evidence related to its development, ecology, and behavior (Dayrat 2005).

For the taxonomic classification of plants, molecular data can support valuable information (Das et al. 2008). The use of DNA markers can solve the limitations of classical taxonomy and the unavailability of reproductive characters. It can be applied at any stage and cannot be influenced by the environment. As reported by Zhu et al. (2014), the sequence-related amplified polymorphism (SRAP) markers are more efficient to differentiate 13 accessions of bamboo in comparison with 22 key morphological characters studied.

16.4 Application of Different Molecular Markers for Bamboo Taxonomy

To overcome the limitations of morphology-based bamboo taxonomy and identification, the inclusion of molecular marker-based technology is the latest solution. There are different types of DNA markers for identification and diversity study. Broadly three types of molecular markers are available: (1) hybridization-based marker (restriction fragment length polymorphism (RFLP)), (2) PCR-based marker (random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP)), and (3) sequence-based marker (single-nucleotide polymorphism (SNP), diversity array technology (DArT)).

From time to time with the available resources, different bambusiasts have included the molecular markers for bamboo taxonomy. As discussed earlier, Attigala et al. (2016) included *Arundinaria wightiana*, a South Indian woody bamboo under the genus *Kuruna* based on morphology. Because of the similarities between the climatic condition of the Western Ghats of India (original habitat of the species) to Sri Lanka and its similar habitation to other species under the genus, reassignment of *Arundinaria wightiana* to *Kuruna* was supported. But, as opined by the author, this species needs further study based on the molecular analysis for confirmation. This section deals with the application of various molecular markers to explore the bamboo systematics.

16.4.1 Restriction Fragment Length Polymorphism (RFLP)

Friar and Kochert included RFLP in 81 species of bamboo during two consecutive studies in (1991) and (1994). In the first study, they constructed *the PstI* library of random probes from *Phyllostachys nigra* genome and screened 61 accessions of temperate bamboo. In another study, they evaluated genetic variation among 20 *Phyllostachys* species. Their study showed that RFLP can be fruitfully used for the identification of species and species demarcation. A hybrid approach was taken by Sen and Goyal (2014) for a diversity study of 29 accessions of bamboo from.

North Bengal, India. They did the study through PCR-RFLP based on the *trnL-F* region. The *trnL*-F region was amplified using specific primer pair and then digested with *Taq*I, *Alu*I, and *Hinf*I. Their study suggested that PCR- RFLP can be used as a tool for phylogenetics screening. Konzen et al. (2017) employed a modified technology of RFLP, i.e., RAPD-RFLP, to study variation among four genera of bamboos. Their study involved digestion of RAPD products (PCR products with RAPD primers) with three different combinations of restriction enzymes. The result showed significant variation among the genera studied and suggested the usefulness of this alternative technique for diversity study at generic level. The study of RFLP in bamboo is limited to date due to its hazardous and time-consuming nature.

16.4.2 Random Amplified Polymorphic DNA (RAPD)

RAPD is a low-cost, easy, and fast marker (Belaj et al. 2001; Deshwall et al. 2005) utilized in the analysis of the phylogenetic relationship among different species, since its discovery by Williams et al. (1990). It requires a high level of polymorphism among species with less quantity of genomic DNA (Williams et al. 1990). Different research conducted by Ko et al. (1998) exhibited RAPD is a fast and responsive technique for polymorphism. As reported by several other workers, RAPD in plant diversity study has significance (Kapteyn and Simon 2002; Welsh and McClelland 1990). In this method, the genomic DNA is amplified with a random decamer primer which binds to DNA on two different sites in opposite direction and amplified if priming sites are within amplifiable distance. The polymorphisms between two species resulted from differences in sequences on one or both of the primer annealing sites and are represented as the presence or absence of a particular RAPD band. It does not require any prior knowledge of sequence information as a result of which it was a marker of choice to many scientists until the draft genome of bamboo came in 2013 (Peng et al. 2013).

Many bambusiasts have employed RAPD for phylogenetic relationship study and identification of bamboo. Lai and Hsaio (1997) employed 13 RAPD markers to characterize and distribute different clones of the *Phyllostachys pubescens* grown in Taiwan. Out of 170 samples collected around the island, 9 clones were identified and the findings suggested very inadequate genetic variation, and the center of variation

happened to be the first region on the island of the successful introduction of the species from China mainland. In the year 2003, Nayak et al. utilized 30 RAPD primers to estimate genetic diversity among 12 bamboo species. Bhattacharya et al. (2006) used 30 decamer RAPD primers from Operon technology for characterization of 17 different geographically different accessions of *B. tulda* in West Bengal. RAPD was also employed to explore phylogenetic relationships among 15 South-eastern China species of *Bambusa* which were placed under several other subgenera (Sun et al. 2006). Their findings suggested that homoplasious floral character-based traditional classifications of woody bamboos require comprehensive evaluation. In the next year, Das et al. (2007) evaluated the phylogenetic relationship among 15 bamboo species from the Botanical Survey of India using morphological and molecular markers. Their study based on molecular parameters (RAPD) was in full compliance with the classical bamboo taxonomist Gamble (1896), though the cluster developed based on qualitative and quantitative morphological traits varied greatly. Ramanayake et al. (2007) estimated genetic diversity among 9 species of bamboo that belong to 4 genera from Sri Lanka by using 41 RAPD primers. Their study showed that RAPD analysis is a potential technique determining genetic diversity as well as solving problematic generic assignment. A gregarious flowering bamboo, *Thamnocalamus spathiflorus* subsp. *Spathiflorus*, was characterized using 30 RAPD primers (Bhattacharya et al. 2009). Lalhruaitluanga and Prasad (2009) estimated genetic diversity among 12 *Melocanna baccifera* accessions from Mizoram by using RAPD markers.

Shalini and his coworkers evaluated the genetic diversity of ten bamboo species using morphological traits along with DNA markers (Shalini et al. 2013). They applied 21 RAPD primers to distinguish the genotypes and found a wide range of genetic variability among the species. Thirty RAPD primers were utilized for the evaluation of phylogenetic relationships among 13 genotypes of Indian bamboo (Desai et al. 2015). Goyal and Sen (2015) studied the phylogenetic relationship using 30 RAPD primers among the 29 bamboo species growing in North Bengal which they have already encountered and studied their distribution pattern (Goyal et al. 2012). Hafzari et al. (2019) evaluated RAPD markers for the identification of five bamboo genera from Indonesia. A total of 25 species from 5 genera were collected for analysis, and their findings suggested RAPD as a useful marker for the diversity study of bamboo. Makmur et al. (2020) very recently conducted a study on eight different types of bamboos from Indonesia. They utilized 20 RAPD primers for genetic diversity evaluation of collected bamboo species. Their result showed RAPD markers successfully estimated genetic diversity among the studied bamboo groups.

16.4.3 Sequence Characterized Amplified Region (SCAR)

SCAR is a fragment of genomic DNA present on a defined genetic locus which is amplified using a specific pair of oligonucleotide primers. Paran and Michelmore

(1993) derived SCAR by sequencing the polymorphic RAPD band of interest and used two ends of the amplified products as primers. SCARs have advantages over RAPD primers as they can amplify a single locus and developed into a codominant marker (Das et al. 2005). SCAR marker with all its advantage is useful for the identification of bamboo species and can resolve taxonomic discrepancies, but to date, the number of SCARs is limited in bamboo.

Das et al. (2005) developed SCAR markers specific for two bamboo species. Their work involved initial screening of 30 random decamer primers to identify loci that are species explicit. Bb_{836} for *B. balcooa* were obtained from random primer PW-02, and Bt_{609} for *B. tulda* were obtained from OPA-08. They validated those two markers with a large number of samples collected from different ecogeographical regions of West Bengal. Another work was reported by Rangsiruji et al. (2018), on the development of SCAR markers in *the Dendrocalamus*. They initially screened 50 RAPD primers in 8 different species of *Dendrocalamus*, and 5 primers showed species-specific loci. Finally, they derived five species-specific SCAR markers which showed potential for identification of five species of *Dendrocalamus*.

16.4.4 Inter Simple Sequence Repeat (ISSR)

ISSRs are regions between two microsatellites or simple sequence repeat regions. The primer is designed for a microsatellite sequence along with two to four arbitrary bases, often degenerate nucleotides at 3′ or 5′ end to amplify the ISSR regions (Qian et al. 2001). The length of the primer is usually 16–25 nucleotides, and thus the annealing temperature is higher than RAPD (decamer primer) making the marker higher reproducible than RAPD. Also, the primer designed from microsatellite regions is unique and distributed across the entire genome which amplifies more molecular allele in comparison with RAPD (Saha et al. 2016). ISSR is considered as the choice of marker for bamboo diversity and phylogenetics relationship study as it does not require any prior knowledge of gene sequence information. Many bambusiasts employed ISSR along with RAPD for genetic diversity assessment in bamboo (Lalhruaitluanga and Prasad 2009; Shalini et al. 2013; Desai et al. 2015; Goyal and Sen 2015).

Lin et al. (2009) utilized 16 ISSR primers along with 15 pairs of AFLP primers for genetic similarity assessment among *Phyllostachys pubescens* cultivars. Statistical analysis showed that a significant correlation existed between the two similarity matrixes obtained from two molecular marker systems. Their study also showed these two molecular markers could prominently identify ten cultivars of *P. pubescens.* In the next year, Lin et al. (2010) utilized ISSR markers for the identification of hybrids produced by crossbreeding of *Phyllostachys* species. Mukherjee et al. (2010) evaluated the genetic relationship with 12 ISSR primers among 22 bamboo taxa. The dendrogram and principal coordinate analysis showed conformity with earlier reports with few exceptions. Their findings revealed that

species of one genus were clustered with members of another genus, which suggests correct delineation of genus and species based on morphology (both vegetative and reproductive features) and inclusion of molecular data along with morphology for classification. Genetic diversity was evaluated among 12 populations of *Dendrocalamus membranaceus* in Yunnan, China, using 10 ISSR primers (Yang et al. 2012). Their findings revealed that the genetic differentiation among the population is significant and no significant correlation exists between genetic and geographical differences among the population tested. Tian et al. (2012) investigated seven populations of *Dendrocalamus giganteus* using seven inter simple sequence repeat primers for the assessment of genetic diversity as the prologue to an effective breeding program.

Seven ISSR markers were applied to analyze genetic variability among six cultivated bamboo species in the Gujrat region (Chaudhary et al. 2015). Nilkanta et al. (2017) investigated the genetic diversity of *Melocanna baccifera*, an economically important bamboo species of Northeast India, by using five ISSR markers. They have conducted the study in seven populations sampled from five districts of Manipur. ISSR marker analysis revealed high within-population genetic variation but low genetic diversity between populations. The result revealed the urge for preservation and protection of all the natural bamboo population in the Northeast region. Amom et al. (2018) employed 10 ISSR primers to investigate the phylogenetic relationship among 15 different bamboo species. Dendrogram analysis and principal component analysis revealed the genetic relationship of 15 bamboo species was in agreement with traditional classification with minor deviations. Ely et al. (2019) studied ecophysiology and genetic diversity of *Chusquea* bamboo species from Venezuela. Genetic diversity study includes ISSR and RAPD markers. Both the marker system showed genetic variation, but ISSR showed higher genetic diversity than RAPD markers, thus suggesting better marker system for genetic diversity study in bamboo. Rajput et al. (2020) applied ISSR along with start codon targeted (SCoT) marker for clonal identification of the tissue culture raised plantlets of *Bambusa balcooa*. In a study by Oumer et al. (2020) in Ethiopia genetic diversity, population structure and gene flow analysis of lowland bamboo (*Oxytenanthera abyssinica*) was conducted using ISSR markers. Their study successfully evidenced that the genetic diversity of the lowland bamboo is associated with geographic locations. A recent report of Amom et al. (2020) witnessed the potential of molecular markers to study the bamboo systematics wherein ISSR and three other DNA markers were used for five native and economically significant bamboo species. Their study revealed that the cluster resulting from phytochemical analysis coordinated strongly with the dendrogram generated from DNA markers suggesting the possibility of combining molecular and phytochemical approaches for genetic relationship study.

16.4.5 Simple Sequence Repeat (SSR) or Microsatellite

Microsatellites are a repeat sequence of one to six nucleotides and abundantly distributed across the eukaryotic genome (Morgante et al. 2002). Microsatellite or SSR markers are characterized by codominant inheritance, reproducibility, high genome coverage, and random dispersion and with a provision to automation through high-throughput genotyping (Lin et al. 2014). The number of SSR markers in bamboo was limited due to the lack of genome information until 2013. Development of SSR in bamboo was both time- and cost-consuming (Chen et al. 2010b). Nayak and Rout (2005) characterized six microsatellites in *Bambusa arundinacea* which were cross-amplified in other bamboo species. Kaneko et al. (2008) isolated and characterized nine SSR markers from *Bambusa arnhemica*. They have suggested that the SSR markers will be useful for investigation of gene flow, evolution, clump characters, and biogeographic history of endemic *B. arnhemica*. Kitamura et al. (2009) isolated ten polymorphic SSR markers from dwarf bamboo *Sasa cernua* and *Sasa kurilensis* and confirmed their applicability in open-pollinated seeds and leaf samples from the natural population. Later Kitamura and Kawahara (2009) investigated the distribution of clones to determine the genetic nature of sporadic flowering in a flowering patch of *Sasa cernua* using eight microsatellite markers developed in their earlier study. Miyazaki and coworkers in the same year developed eight polymorphic SSR markers from *Sasa senanensis* and investigated cross transferability in other dwarf bamboos. Moreover, the cross transferability of rice SSR markers was tested in bamboo, and 120 SSR markers from rice were evaluated in 21 species of bamboo (Chen et al. 2010b). Out of 120 markers, 82 amplified successfully in bamboo genotype, and SSR markers positioned on rice chromosome 7 and chromosome 1 exhibited, respectively, the highest and the lowest transferability. Their study demonstrated rice SSR can be successfully utilized for diversity study in bamboo. Tang et al. (2010) examined the available public domain sequence database and analyzed 1532 *Phyllostachys pubescens* sequences. They discovered 3241 SSR loci of di- or more nucleotide repeat sequences in 920 genomic and 68 cDNA sequences. They developed a total of 19 microsatellite markers and checked for cross transferability in 6 other *Phyllostachys* species. All the markers were transferred successfully in all six species of *Phyllostachys* and showed high polymorphism. Sixteen novel microsatellite markers were developed in the strongest woody bamboo, *Dendrocalamus sinicus*, using Fast Isolation by AFLP of Sequences COntaining Repeats (FIASCO) protocol (Dong et al. 2012). The SSRs developed were successfully cross-amplified in other *Dendrocalamus* species and thus signified that the markers can be employed in diversity analysis of the *Dendrocalamus*.

Twenty microsatellite markers were developed by Jiang et al. (2013) for *Phyllostachys edulis*, an economically important bamboo species of China. They have tested those 20 markers on 71 samples collected from 3 geographically isolated regions. Each marker produced between two and ten amplicons and will support in future studies on different aspects of *P. edulis* like molecular ecology, conservation,

etc. Full-length cDNA (FL-cDNAs) databases give a prosperous resource for developing potential FL-cDNA SSR markers. Lin et al. (2014) screened 10,608 cDNAs of *Phyllostachys pubescens* and discovered 1614 SSRs in 1382 SSR-containing FL-cDNAs. The applicability was confirmed by using the FL-cDNA SSR markers to spot the parental stock in interspecific hybrids of bamboo. Later, Zhao et al. (2015) physically mapped 1098 microsatellites on the Moso bamboo (*Phyllostachys edulis*) genome and validated 917 markers in 9 accessions with ~39.8% polymorphisms. They have implemented a database for bamboo microsatellite (http://www.bamboogdb.org/ssr). The markers developed are valuable for the study of molecular marker-based taxonomy in bamboo. Very recently Rossarolla et al. (2020) have characterized ten polymorphic SSR markers of *Guadua chacoensis* and also successfully cross-amplified in other species of bamboo.

16.4.6 Expressed Sequence Tagged-Simple Sequence Repeat (EST-SSR)

Nowadays online databases are a great source of sequence information. The EST sequence project for the discovery of genes in several plants generated a huge amount of DNA sequence data deposited in online databases (Rudd 2003). The public domain databases can be accessed with some specific computer programs and can be searched for SSR motifs, which are known as genic or EST-SSR microsatellites. The genic SSRs or EST-SSRs are limited to those species or closely related species for which large amounts of ESTs are available and submitted (Varshney et al. 2005).

At the beginning of the twenty-first century, gene sequence data was scarce in bamboo, so EST-SSR in bamboo was unavailable. However, few bambusiasts employed EST-SSR study in bamboo from other cereal crops such as Barkley et al. (2005) and Sharma et al. (2008). Barkley et al. (2005) used 25 EST-SSR markers from maize, wheat, sorghum, and rice and checked transferability in bamboo. Ninety-two accessions of bamboo belonging to 11 genera and 44 species were studied for genetic diversity study. Sharma et al. (2008) utilized 20 EST-SSRs, developed from the sugarcane genome, in assessing genetic distances among 23 bamboo species. Their major findings indicated EST-SSRs from cereal crops can be successfully utilized in bamboo for diversity study.

In the subsequent year, Sharma et al. (2009) mined 329 ESTs of *Bambusa oldhamii* and *Phyllostachys edulis* in public sequence databases and using different computer programs identified 10 successful EST-SSR markers from *B. oldhamii* ESTs. Their cross transferability level was higher and amplified consistently in other species suggesting their usefulness in diversity study as well as in genetic analyses of bamboo species. Dong et al. (2011) searched 3406 publically available ESTs of *Bambusa oldhamii* and *Phyllostachys edulis* and discovered 245 nonredundant SSR markers in 205 EST contigs and developed 15 EST-SSR markers. The transferability

of those markers was checked in 14 caespitose bamboo species and 2 markers, viz., BOM01 and BOM02, transferred to almost all the caespitose bamboos producing species-specific alleles which could be utilized for identification of caespitose bamboo interspecies hybrids. Bhandawat et al. (2019) mined 8121 EST-SSR markers from *Dendrocalamus hamiltonii* transcriptome data, and they developed a set of 114 polymorphic markers which are linked with several biogenic factors such as transcription factors, cell cycle regulators, signaling, etc. Genetic diversity and population structure were evaluated among 72 accessions belonging to three populations of *D. hamiltonii*. Cai et al. (2019) identified 18,356 EST-SSR loci from *Phyllostachys violascens* transcriptomic data. A total of 11,264 primer pairs were designed, and a total of 96 primer pairs were selected randomly and synthesized. Out of 96 primers synthesized, 54 were used to study variation among 16 *P. violascens* bamboo and 10 other species of *Phyllostachys*. Their study generated rich EST-SSR markers for genetic diversity study in bamboo.

16.4.7 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a robust, reliable genetic marker discovered by Vos et al. (1995) and shows a significant level of DNA polymorphism. Loh et al. (2000) conducted a study using AFLP markers for genetic variation and relationship study in four bamboo genera under the Bambusinae subtribe. AFLPs discriminated against different species understudy with a unique banding profile. Unique AFLPs were detected in 13 out of the 15 bamboo species studied. To explore the clonal structure of a dense population of dwarf bamboo, *Sasa senanensis* AFLP profiling was used (Suyama et al. 2000). AFLP fingerprinting of 51 *S. senanensis* population from a study plot in Japan suggested that the plot was consisted of at least 22 clones. A comparative study on two contrasting molecular techniques, viz., AFLP and ITS-nrDNA, for phylogenetic relationship assessment of *Phyllostachys* bamboo was conducted (Hodkinson et al. 2000). Twenty-two species of *Phyllostachys* were considered for the study and the 5S spacer region of nrDNA investigated along with two selective AFLP markers. Their result showed that AFLP analysis exhibited a higher degree of discrimination, and the 5S spacer region is unsuitable for this purpose. AFLP was suggested as the better choice of marker for phylogenetics relation study. Marulanda and coworker utilized AFLP markers to describe the association between accessions and biotypes of *Guadua angustifolia* and compared them with other *Guadua* species of Columbia (Marulanda et al. 2002). Fifty-five accessions were studied using three combinations of primer, and a clear genetic difference was observed between the different species of the *Guadua* genus.

Molecular marker-based identification of clonal plants is superior to other techniques, but in this process also there are two limitations: first, wrong identification of genetically similar seedlings as clones, and secondly, wrong identification of two same clones having different fingerprints as genetically different individuals. The problem was addressed by Douhovnikoff and Dodd (2003) in *Salix exigua* using the

higher precision of differentiation of AFLP fingerprint and developed the threshold value of Jaccard's similarity index (0.983) for assigning individuals to clones. The result showed the approach was useful in the precise identification of clones. The flowering incident in bamboo is itself an interesting phenomenon as the flowering cycle varies greatly in different species of bamboo. The flowering cycle of *Phyllostachys pubescens* was calculated precisely as 67 years. In a community of *P. pubescens*, the flowering and nonflowering culms were mixed, and flowering episodes lasted as long as 3 years in the population. AFLP analysis by Isagi et al. (2004) confirmed separate stands or genets of *Phyllostachys pubescens* that originated from an earlier flowering incident. AFLP fingerprint technology was utilized by Mathews et al. (2009) for the clonal diversity assessment of *Arundinaria gigantea* in Western North Carolina. Their study helped in the restoration project of *A. gigantea* by identifying the clonal diversity in different stands and cause of culm loss. The genetic structure of *Sasa pubiculmis* was identified, and the flowering pattern along with the seed set was investigated using the AFLP technique (Miyazaki et al. 2009).

Phylogenetic relationship as well as the genetic variability based on AFLP markers among edible bamboos from Northeast India (Ghosh et al. 2011) and landraces of *Dendrocalamus hamiltonii* (Waikhom et al. 2012) were evaluated. In a different study by Lin et al. (2011a, b), the efficiency of the AFLP marker for genetic diversity assessment was established in *Phyllostachys violascens*. ISSR, sequence-related amplified polymorphism (SRAP), and AFLP techniques were used for the evaluation of phylogenetic relationships within different cultivars of *Phyllostachys violascens*. Their findings demonstrated that all three marker system were useful for genetic diversity estimation in *P. violascens*, though AFLP was the most resourceful marker. Waikhom et al. (2012) further tested the four pairs of markers through multiple regression analysis for marker-trait association identification, and a positive correlation was found between AFLP data and biochemical attributes, i.e., antioxidant activity and total cyanide content. Eight AFLP primers along with 42 RAPD primers were utilized for genetic diversity assessment of industrially important red bamboo, *Ochlandra travancorica*, from Kerala, India (Nag et al. 2013). A relatively high amount of polymorphism was observed which could be useful in the selection of elite germplasm for improvement.

16.4.8 DNA Barcoding and Molecular Phylogeny in Bamboo

A short stretch of DNA sequence from a standardized region of the genome which can be utilized uniquely for identification of the species is termed as DNA barcode. The concept of using DNA barcode for species identification was proposed by Hebert et al. (2003) using mitochondrial cytochrome *c* oxidase (*COI*) barcode region in the animal. In the case of plants, the *COI* gene and other mitochondrial regions are not useful barcode region for identification of species due to its low genetic variation and variable structure of the mitochondrial genome (Kress et al. 2005; Chase et al.

2005; Pennisi 2007; Chase et al. 2007; Fazekas et al. 2008). Consortium for the Barcode of Life's (CBOL) Plant Working Group (2009) suggested two locus combinations of *rbcL*+ *matK* as a potential DNA barcode for plant species identification. The nuclear ribosomal -*ITS*2 region is recommended as a potential tool for the identification of plant taxa (Chen et al. 2010a). Several studies reported different regions of plastid DNA alone or in combination as potential barcode regions in plants, viz., *trnH-psbA* (Kress et al. 2005), *rpoC1* + *rpoB* + *matK* or *rpoC1* + *matK* + *trnH-psbA* (Chase et al. 2007), and *rbcL* + *trnH-psbA* (Kress and Erickson 2007). DNA barcoding is a helpful tool for taxonomic classification and recently gaining preference over classical taxonomy due to its accuracy in identification (Sijimol et al. 2014).

Sequence-based phylogenetic relationship study in bamboo was conducted as early as in (2005) by Qiang et al. They applied nrDNA *ITS* region and cpDNA *trnL-F* intergenic spacer for genetic relationship study of *Arundinaria* and related genera. The sequence-based phylogenetic tree was inconsistent with the morphological character-based tree. In the same year, Sun et al. (2005) employed a nuclear rDNA *ITS* sequence for phylogenetic analysis of *Bambusa*. Their study raised the question about the monophyly of the different subgenera under the *Bambusa* genus. Yang et al. (2008) employed nuclear rDNA *ITS* and GBBSI gene along with a plastid *trnL-F* spacer sequence to assess the phylogenetic relationship and fruit evolutionary analysis. Their study suggested reorientation at subtribe level and fruit characters are not reliable for phylogeny. The study further reveals bacoid caryopsis may represent particular ecological condition-based specialization. Ruiz-Sanchez and Sosa (2010) utilized molecular data along with morphological and ecological data for delimiting species boundaries within the Neotropical bamboo *Otatea*. They employed cpDNA regions *atpF-atpH*, *psbK-psbI*, and *trnL-rpl32* in combination and nrDNA internal transcribed spacer (*ITS*) region. Their result assigned seven species under *Otatea* instead of three as previously described.

Cai et al. (2012) conducted a study for testing four candidate barcode markers, viz., *matK*, *rbcL*, *psbA-trnH*, and *ITS2*, in temperate woody bamboos. The study revealed *rbcL* + *ITS2* as the potential marker combination for the identification of temperate woody bamboos. The core barcode *matK* failed to identify *Bambusa* species due to polyploidization, interspecies hybridization, and introgression (Das et al. 2013). Sosa et al. (2013) worked on 36 species of bamboo and evaluated the efficiency of *rbcL*, *matK*, and *psbI-K* spacer region individually and in combination. Their study revealed that *matK* + *psbI-K* were able to identify the woody bamboos at the generic level. Ghosh et al. (2017) worked on 21 tropical bamboo species and reconstructed phylogeny based on *ITS1* and *ITS2* sequence and their consensus secondary structure. Their findings reveal that the sequence alone could reconstruct the traditional phylogeny, but few inconsistencies were found and they integrated the secondary structure of the *ITS* sequence which helped in the resolution of the tree. Their study suggested a combination of the structure along with sequence for phylogenetic relationship assessment of bamboo. Tyrrell et al. (2018) employed

four plastid markers, viz., *ndhF*, *trnC-rpoB*, *trnD-trnT*, and *rps16-trnQ*, for phylogenetic analysis of 31 Neotropical woody bamboos under the genus *Arthrostylidium*. Their molecular phylogenetic study along with leaf anatomy study revealed that three species under the abovementioned genus do not belong to them, but rather they represent a different genus under the subtribe Guaduinae and they erected a new genus *Tibisia*. CBOL-recommended seven standard barcode regions were evaluated for the detection of potential barcode for commercially important bamboo species identification (Dev et al. 2020). Their study suggests *psbA-trnH* DNA barcode region can be utilized to spot the species and identify the planting materials of their bamboo.

16.5 Conclusions

Bamboo classification based on traditional taxonomic analysis is tedious and sometimes misleading. The classification based on floral characters is limited due to long flowering cycles, and that may also be homoplasious. So, for taxonomic classification, bambusiasts depend on vegetative characters which are frequently influenced by the environment. The molecular marker-based taxonomic classification is a step forward for confirmation and solving taxonomic discrepancies as well as for delineation and identification of bamboo species. As evident, various molecular markers have been successfully utilized for the characterization of bamboo species. For solving problematic generic assignment, the utilization of RAPD, ISSR, SSR, and AFLP, as a sole and/or in combination, was very successful in this species. The results, so obtained, may also be very useful for preserving and protecting the natural bamboo populations. Besides, SSR and/or EST-SSRs have the potential for the investigation of clump structure, the evolution of the bamboo flowering signal, models of gene flow, ecology, population structure, the biogeographic history, and conservation of endemic bamboo species. The high level of cross transferability of SSRs and reliable amplification in other species suggested their utility in diversity study as well as in functional and genetic analyses of bamboo species. SCAR marker with all its advantage is useful for the identification of bamboo species and can resolve taxonomic discrepancies. DNA barcoding is a robust and reliable tool for taxonomic classification and recently gaining preference over classical taxonomy due to its accuracy in the identification of candidate barcode markers. Among the different available barcode region, nrDNA *ITS* in combination with cpDNA *rbcL* and *psbA-trnH* spacer proved as a potential barcode for bamboo identification. DNA markers combining with morphological and phytochemical approaches are suggested to use for proper characterization, exploring the genetic property, and studying genetic relationship which can reconstruct the phylogenetic tree with further resolution among bamboo species.

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