Chapter 10
Polymorphism and Phylogenetic Relationships in Bamboo

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Abstract A breakthrough in plant systematics began to develop at the end of the nineteenth century, since the development of molecular systematics. This method is considered to contribute to supporting the phylogenetic framework in the plant world. Molecular studies are expected to strengthen existing systematics, not replace them. Until the late 1980s, the bamboo classification system was still based on morphological data. In the early 1990s, identification was started using molecular markers. This identification can provide important information in overcoming various taxonomic constraints. It can determine the taxon level of a type appropriately and corresponds to taxonomic data based on morphological characters. Scientists use various molecular markers to look for similarities or differences between species. Some of the molecular markers used are amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCARs), start codon targeted (SCoT), inter-primer binding site (iPBS), and simple sequence repeats (SSR). In addition to molecular markers, bamboo taxonomy is also carried out using DNA sequence-based methods. This method includes sequences of organelle genes and nucleus genes. Furthermore, several chloroplast genes were also found to form molecular relationships in Poaceae. This chapter is aiming to provide a piece of up-to-date information on molecular markers applied in different bamboo species to evaluate the genetic relationships.

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10.1 Introduction

Gregor J. Mendel put forward the idea of the marker during his experiment back in the nineteenth century. He used phenotype-based genetic markers. Again, during experiments on *Drosophila*, phenotype-based genetic markers were used that led to the establishment of theory of genetic linkage (Agarwal et al. 2008). Although these phenotypic-based markers contributed very much in the past, it has certain drawbacks which limit their use in present time. The foremost limitation is the changing environmental conditions which acutely influence and more importantly cover a limited portion of plant genome which is their major drawback (Amom and Nongdam 2017). These limitations caused the invention of more beneficial markers based on DNA which are now known as molecular markers. A molecular marker is demarcated as a specific unit of DNA that is representative of the variances at the genome level. Also, it is not necessary that molecular markers correlate with phenotypic expression of a trait (Agarwal et al. 2008). The idea of DNA-dependent markers has improved our capability several times in understanding even a minute section of the chromosome. Molecular markers are used for various purposes such as genetic variability evaluation, genome fingerprinting genetic, physical mapping of genomes, population genetic studies, and marker-assisted breeding for crop enhancement. A list of molecular markers has been given in Table 10.1. In spite of the advancement in genome sequencing technologies, molecular markers remain to continue to be a crucial mechanism for extensive analyses of the genome, not solely by enabling assembly of the genome but through their verified worth in high-throughput genotyping, comparative and evolutionary genomics, trait mapping, and breeding in plants. Molecular markers find even the slight variation like a single-base variation in the genome which makes them beneficial in detecting DNA polymorphisms usually related to desirable traits and also in detecting and analyzing involved alleles (Hayward et al. 2015).

The characteristic of a perfect marker comprises of having a highly polymorphic nature, codominant inheritance, and a regular presence in the genome, remaining the same in changing environmental condition, and being easily accessible and highly reproducible (Ibrahim et al. 2010). The best-suited marker can select according to their physical characteristic and location in genome, the cost required, the comfort during use, and the amount of throughput essential (Hayward et al. 2015). The primary marker technique that was used for the physical mapping of plant genomes was RFLP. The method is costly and relies on previous sequence data. After the discovery of PCR technique-based marker systems such as RAPD, AFLP, AP-PCR, etc., the existing strains were relieved. These markers are quick and cheap and do not need knowledge of previous sequences. Methods such as RAPD and AFLP are helpful in population genetics study and breeding resolutions. They are also helpful in tagging a phenotypic trait to a genetic component. SCAR system was intended for

Table 10.1 List of molecular markers

Markers	Name	Reference
AFLP	Amplified fragment length polymorphism	Vos et al. (1995)
AMP-PCR	Anchored microsatellite primed PCR	Wolf et al. (1995)
CAPS	Cleaved amplified polymorphic sequence	Michaels and Amasino (1998)
DALP	Direct amplification of length polymorphism	Desmarais et al. (1998)
ASSR	Anchored simple sequence repeats	Wu et al. (1994)
DAMDPCR	Direct amplification of microsatellite DNA by PCR	Heath et al. (1993)
ASA	Allele-specific amplification	Wu et al. (1989)
DArT	Diversity array technology	Jaccoud et al. (2001)
IRAP	Inter-retrotransposon amplified polymorphism	Kalendar et al. (1999)
SSR	Simple sequence repeats	Litt and Luty (1989)
SSAP	Sequence-specific amplification polymorphism	Waugh et al. (1997)
VNTR	Variable number of tandem repeat	Jeffreys et al. (1985)
SPAR	Single primer amplification reactions	Gupta et al. (1994)
STAR	Sequence-tagged amplified region	Rafalski and Tingey (1993)
SSCP	Single-strand conformational polymorphism	Hayashi (1992)
SNP	Single-nucleotide polymorphism	Landegren et al. (1988)
IM-PCR	Inter-microsatellite PCR	Zietkiewicz et al. (1994)
AP-PCR	Arbitrarily primed PCR	Welsh and McClelland (1991)
DAF	DNA amplification fingerprinting	Caetano-Anolles et al. (1991)
IFLP	Inter-fragment length polymorphism	Hongtrakul et al. (1998)
MP-PCR	Microsatellite-primed PCR	Meyer et al. (1993)
MAAP	Multiple arbitrary amplicon profiling	Caetano-Anolles and Gresshoff (1994)
RAHM	Random amplified hybridizing microsatellites	Ciffarelli et al. (1995)
REM	Retrotransposon microsatellite amplified polymorphism	Kalendar et al. (1999)
SCAR	Sequence characterized amplified regions	Michelmore et al. (1991), Martin et al. (1991)
SSLP	Simple sequence length polymorphism	Tautz (1989)
RBiP	Retrotransposon-based insertion polymorphism	Flavell et al. (1998)
OLA	Oligonucleotide ligation assay	Landegren et al. (1988)
RAM	Random amplified microsatellites	Ender et al. (1996)
REMAP	Retrotransposon microsatellite amplified polymorphism	Kalendar et al. (1999)
SAMPL	Selective amplification of microsatellite polymorphic loci	Morgante and Vogel (1994)
STMS	Sequence-tagged microsatellite site	Beckmann and Soller (1990)

(continued)

Table 10.1 (continued)

Markers	Name	Reference
STR	Short tandem repeats	Edwards et al. (1991)
ISSR	Inter-simple sequence repeat	Zietkiewicz et al. (1994)
RAMP	Random amplified microsatellite polymorphisms	Wu et al. (1994)
RAPD	Random amplified polymorphic DNA	Williams et al. (1993)
RFLP	Restriction fragment length polymorphism	Friar and Kochert (1994)

converting arbitrarily primed PCR products into genomic physical landmarks. Other markers such as microsatellite marker technology exploit the intraindividual and interindividual difference in microsatellites or SSR region for analyzing the fingerprint. For delineating the parental lineage, chloroplast and mitochondrial microsatellite- dependent markers are used which in turn give the best results during breeding and crop improvement (Agarwal et al. 2008). With quick advancement in technique of molecular biology, much effective and greater markers may develop in coming times which can significantly accelerate research in plant breeding.

Almost all molecular methods have become now a basic necessity in the key finding of biological science. Similarly, molecular approaches such as variable number tandem repeats (VNTRs), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) have become an important part of the genetic diversity assays. However, it is very important to understand that different markers have different properties, so it's inevitable that the markers would show different results as well (Karp and Edwards 1995) (Table 10.2). Moreover, molecular data could help us to deal with the more complex questions like taxonomy of the plant (Das et al. 2008). Molecular approaches help us to study the evolutionary phases of a plant and relative diversity of a species (Nayak et al. 2003). Loh et al. (2000) reported that till 2000, the application of molecular approaches in the field of genetic diversity particularly in bamboo was limited. Two types of molecular markers are mostly used in order to study the genetic diversity: (1) hybridization-based, i.e., RFLP, and (2) PCR-based, i.e., AFLP, RAPD, SSR, inter-simple sequence repeats (ISSR), and single-nucleotide polymorphism (SNP). RFLP markers have been reported to be showing a low level of polymorphism as compared to others. Friar and Kochert (1994) employed RFLP for almost 20 *Phyllostachys* species to study genetic variation and evolution. Moreover, it needs a fine quality of DNA. Similarly, RAPD is very easy to use as it does not need any information of plant genome before the application, and this feature has made its use for a large number of plants in order to study the genetic diversity among the species or within the species, whether on basic of ecological or geographical factors (Belaj et al. 2001; Deshwall et al. 2005).

The characters that are commonly used for the identification and classification of plants are morphological, cytological, phytochemical, anatomical, ecological, physiological, and molecular (Stace 1989; Singh 2010) (Table 10.3). The generative

Table 10.2 Application of different markers to study polymorphism and phylogenetic relationship in bamboo

Markers used	Species name	Remarks	Reference
RAPD, ISSR, iPBS, SCoT RAPD	*Bambusa cacharensis*, *B. mizorameana*, *Dendrocalamus manipureanus*, *D. hamiltonii*, and *D. sikkimensis*	Ten primers of each markers were used to examine the genetic polymorphism and relationship between 50 genotypes of 5 important bamboos	Amom et al. (2020)
RAPD	*Bambusa* sp.	Phylogenetic relationships among 28 species of *Bambusa* were examined by using 16 RAPD markers	Rong et al. (2020)
RAPD	*Dinochloa*, *Bambusa* sp., *Dendrocalamus*	Genetic relationship has been identified between bamboo species belonging to five genera	Nayak et al. (2003)
RAPD	*Bambusa* sp.	The species of *Bambusa* belonging to southeastern China have been investigated, particularly in order to study their genetic relationship	Friar and Kochert (1991)
RAPD	*Gigantochloa*, *Arundinaria* sp., and *Dendrocalamus*	Reported the genetic distance between genera *Bambusa* and *Gigantochloa*	Sun et al. (2006) Ramanayake et al. (2007)
AFLP	*Dendrocalamus*, *Bambusa* sp., *Bamboo* sp., *Guadua angustifolia*	Phylogenetic and genetic variability among 12 bamboo species belonging to northeastern region of India	Ghosh et al. (2011)
AFLP	*Phyllostachys* sp.	Phylogenetic relationship among bamboo spread out across regions of the world	Kobayashi (1997)
AFLP	*Phyllostachys pubescens*	Analysis of germplasm of *Guadua* particularly in the coffee region of Colombia	Marulanda et al. (2002)
		Phylogenetic studies in genus *Phyllostachys*	Hodkinson et al. (2000)
		Ten cultivars of *P. pubescens* were identified which have a highest degree of similarity	Lin et al. (2009)
ISSR	15 different bamboo species including *B. mizorameana*, *B. manipureana*, *D. sikkimensis*, and *D. manipureanus*	ISSR markers were used to examine the genetic relationship of 15 various bamboo species of Northeast India	Amom et al. (2018)
CpDNA	*Asian bamboos*	CpDNA restriction site mutations were examined in 16 bamboo species of Asia	Watanable et al. (1994)
CpDNA	*Bamboo* sp.	Chloroplast genome sequencing study was conducted	Zhang et al. (2011a)
CpDNA	*Bamboo* sp.	Studied polymorphism and genetic relationship among 22 species of bamboo	Zhang et al. (2011b)

(continued)

Table 10.2 (continued)

Markers used	Species name	Remarks	Reference
SSR	*Guadua chacoensis*, *Merostachys* *G. chacoensis*	Phylogenetic inference and SSR characterization of tropical woody bamboos tribes Bambuseae (Poaceae: Bambusoideae) were carried out on the basis of complete plastid genome sequences Identification and characterization of SSR markers for genetic studies along with the evaluation of their transferability with the other bamboo species were performed	Vieira et al. (2016) Rossarolla et al. (2020)
SCAR	*B. balcooa*, *B. tulda*	Generated SCAR fragments (species-specific)	Das et al. (2005)
MITEs	*B. multiplex* *B. vulgaris*, *Sasa veitchii*	Ac-like sequence was found Ac-like transposon element was found	Huttley et al. (1995) Gielis (1998)
ITS sequences	*Eremitis*, *Pariana*, and *Parianella* *Arundinaria* sp.	The herbaceous bamboos (tribe Olyreae) were analyzed based on ITS and plastid DNA (*rpl32-trnL* and *trnD-trnT* spacers) to establish phylogenetic relationship within Parianinae Phylogenetic relationships were studied between *Arundinaria* and some related genera such as *Bashania*, *Pleioblastus*, *Pseudosasa*, *Clavinodum*, etc. using special ITS sequences like nrDNA	Ferreira et al. (2019) Qiang et al. (2005)
cDNA library	*B. oldhamii*	Few DNA clones that involve in sucrose synthesis such as BoSus1, BoSus2, BoSus3, and BoSus4 were analyzed from etiolated bamboo shoots	Chiu et al. (2006)
RT-PCR and microarray analysis	*Phyllostachys praecox*	Several rhizome genes were studied that involve in differentiation of rhizome into rhizome shoots, rhizome buds, bamboo shoots, leaves, etc.	Wang et al. (2010)

RAPD random amplified polymorphic DNA, *ISSR* inter-simple sequence repeats, *iPBS* inter-primer bonding site, *SCoT* start codon targeted, *AFLP* amplified fragment length polymorphism, *CpDNA* cytoplasmic DNA, *SSR* simple sequence repeats, *SCAR* sequence characterized amplified regions, *MITEs* miniature inverted-repeat transposable elements, *ITS* internal transcribed spacers

Table 10.3 Genetic fidelity testing of in vitro raised bamboos

Type of marker	Species name	Remarks	Reference
Morphological descriptors	*Dendrocalamus asper* *D. hamiltonii*	In vitro raised plants were compared with mother plants but found no significant variation Most of the leaf features were found comparable to the mother plant	Singh et al. (2013) Bag et al. (2012)
Biochemical analysis	*D. hamiltonii*	The chlorophyll pigment and leaf mass of the in vitro raised plants were found to be comparable to the mother plant	Bag et al. (2012)
Physiological studies	*D. hamiltonii*	Similarly, the rate of photosynthesis and the water intake efficiency of the in vitro raised and hardened plant were found to be comparable to the mother plant	Agnihotri and Nandi (2009)
Molecular markers RAPD	*Bambusa balcooa*, *B. tulda.* *D. hamiltonii*	Studied the confirmation of genetic fidelity of in vitro raised plants and further suggested that the axillary meristem is the viable part for clonal propagation Genetic fidelity was reported during various stages of development of in vitro raised plant and confirmed the absence of somaclonal variation	Das and Pal (2005) Agnihotri and Nandi (2009)
ISSR	*B. nutans* *G. angustifolia* *B. Balcooa*	The shoot multiplication up to 24th cycle till to the hardening of the in vitro raised plants grown in polyhouse were found genetically similar to the mother plant Similarly, the genetic fidelity was evaluated till to the hardening phase of the in vitro raised plant and was compared with mother plant The monomorphic banding patter was found to be similar with the mother plant	Negi and Saxena (2011) Nadha et al. (2011) Rajput et al. (2020)
SSR	*D. asper*	Similarly, no somaclonal variation was reported, and the in vitro raised plants were genetically similar to that of mother plant	Singh et al. (2012)
AFLP	*B. balcooa*	The tissue culture grown plants emerging from the axillary buds and somatic embryogenesis were having no any epigenetic changes	Gillis et al. (2007)
SCoT	*B. balcooa*	The monomorphic banding pattern of SCoT marker of in vitro derived plants matched with mother plants confirmed the genetic similarity	Rajput et al. (2020)

RAPD random amplified polymorphic DNA, *ISSR* inter-simple sequence repeats, *SSR* simple sequence repeats, *AFLP* amplified fragment length polymorphism, *SCoT* start codon targeted

organs' nature is more ideal for characterization than the vegetative organs because their structure is constant and provides more properties for the differentiation of taxa. In some taxa, vegetative traits have a low taxonomic value, but for taxa that have a low inflorescence frequency, vegetative traits are essential in classification (Jones

and Luchsinger 1986). Over the years, botanists have laid the foundations of systematics and identification of bamboos based on morphological and anatomical characters. However, the systematics is based on vegetative characters only. The resulting data is less accurate, so it still needs to be compared with data from other analyses (Das et al. 2008).

Bamboo identification in many countries has been carried out using DNA fingerprint methods, such as random amplified polymorphic DNA (RAPD) (Nayak et al. 2003), amplified fragment length polymorphism (AFLP) (Loh et al. 2000), sequence characterized amplified regions (SCARs) (Das et al. 2005), inter-simple sequence repeat (ISSR) (Negi and Saxena 2010), simple sequence repeats (SSRs) (Nayak and Rout 2005), expressed sequence tag-simple sequence repeat (EST-SSR) (Sharma et al. 2009), and transposons (Keukeleire et al. 2004). Furthermore, Das et al. (2008) succeeded in making dendrogram comparisons of bamboo relationships in India based on morphological and molecular characters.

A taxonomic method based on DNA sequences was also developed to determine genetic diversity, population structure, and phylogenetic relationship between bamboo species. Sun et al. (2005) used the internal transcribed spacer (ITS) rDNA sequence for phylogenetic analysis of *Bambusa* in China. The results of ITS rDNA regional sequences ranged from 637 bp in *Guadua angustifolia* to 696 bp in *Bambusa flexuosa*. The similarity values obtained ranged from 86 to 100%. The identification results can show phylogenetic patterns between *Bambusa* species and their close relatives. Meanwhile, Goh et al. (2010) reported that the phylogenetic relationship analysis of bamboo was also carried out using chloroplast DNA *rps16-trnQ*, *trnC-rpoB*, *trnH-psbA*, and *trnD-T*, and nuclear DNA, namely, the *GBSSI* gene. In a recent study of Liu et al. (2020), double-digest restriction site-associated DNA (ddRAD) sequencing was performed to reveal the phylogenetic relationship of the four important genera of *Bambusa-Dendrocalamus-Gigantochloa* complex.

The present chapter aims to provide the information on different molecular markers, for example, DNA fingerprint-based method, DNA sequence-based method, etc., applied on bamboo to establish their genetic relationships. Moreover, a description of the role of morphological characters for the identification of bamboo has also been discussed.

10.2 Morphological Traits: Key to Bamboo Identification and Characterization

Gamble was probably the first scientist who identified bamboo plants on the basis of morphological characters particularly vegetative and reproductive characters in 1896. However, scientists later on discovered other morphological characters such as culm sheaths that became tools for the early classification of bamboos. Finally, Chatterjee and Raizada (1963) set a culm sheath character, a parameter to identify 22 bamboo taxa. According to Chatterjee and Raizada (1963), the culm sheath

characters such as size, texture, blades, and shape of the blade offer a good line of distinction for the classification of bamboos. Similarly, Bennet and Gaur (1990) suggested that the branching pattern could become an important characteristic for the identification of genus. They even suggested that the sprouting vegetative buds could also serve as an important morphological character for the identification of bamboos. Triplett and Clark (2003) have tried to understand the relationship between ecological and geographical variations with the genetic diversity, so they took 7 vegetative and 14 reproductive characters. The principle of their work was that the variations in the characters are a continuous process, so therefore it couldn't act as a kind of parameter to classify the species on the basis of their morphological characters. Their work emphasized the need to conduct more in-depth analysis in order to determine the classification of *C. culeou*. A study was conducted by Das et al. (2007) by means of 32 qualitative and quantitative morphological characters in order to understand the phylogenetic relationship of 15 species of bamboo which were not in agreement with the classification of Gamble (1896).

10.2.1 Limitation of Morphological Characters

The following are some of the limitations that basically guide us that classification on the basis of morphological or vegetative characters would not set a precedent: (1) According to Janzen (1976), the reproductive cycle of the bamboo is too long and that could stretch up to 120 years. So using floral characters for characterization or identification would serve no purpose. (2) Evolutionary studies dictate that the vegetative characters are subjected to environmental impact. So vegetative characters would not be a reliable key for taxonomic classification.

10.3 DNA Fingerprinting-Based Methods

10.3.1 RFLP

The basis of the polymorphisms in the RFLP is the difference in the sequence of the restriction enzyme recognition sites between genomes. This marker is codominant and useful for selection with the help of specific markers. Friar and Kochert (1994) first used RFLP on *Phyllostachys* to study the genetic variability and evolution of its 20 species. This technique is rarely used in bamboo because it requires high-quality DNA and skilled personnel.

10.3.2 RAPD

RAPD is an inexpensive and fast method and does not require preliminary information from the plant genome. This method has been widely used to study plant genetic variation because it is sensitive and effective in obtaining polymorphism data. Random amplified polymorphic DNA (RAPD) molecular markers have been used to reveal that *Bambusa ventricosa* and *B. vulgaris* var. *striata* are the same species (Nayak et al. 2003). The RAPD technique has also separated the spiny *Bambusa* from the *Dendrocalamus* members (Sun et al. 2006). The RAPD technique has also been successful in demonstrating high levels of polymorphism in nine bamboo species in Sri Lanka (Ramanayake et al. 2007). However, the RAPD technique is not suitable for the identification of polymorphisms within species. Bhattacharya et al. (2006) proved that the identification of 17 *B. tulda* populations that experienced geographic isolation did not show any polymorphisms. The same thing happened in the study of Lai and Hsiao (1997), where out of 176 samples of *P. pubescens*, only 9 genotypes were found. These results indicate the genetic diversity in the population is very low.

10.3.3 SCARs

Sequence characterized amplified regions (SCARs) is the development of RAPD (Paran and Michelmore 1993). In the bamboo study, SCARs were used to identify genotypes and varieties, especially for species that have almost the same morphological characteristics. The SCAR marker was developed by Das et al. (2005) for *Bambusa balcooa* and *Bambusa tulda*, in order to assist the paper industry in differentiating the two types of bamboo. Meanwhile, Bhattacharya et al. (2008) conducted a genetic diversity study on 12 populations of *Bambusa balcooa* and 17 populations of *Bambusa tulda* based on morphological characters and molecular marker SCAR in India. The results of these studies indicate a high morphological diversity between *Bambusa* populations. This is known through the coefficient of diversity 49.49% (F value 10.4326; $P = <0.001$). However, the absence of DNA band polymorphisms in SCAR indicates the low intraspecific genetic diversity of the two bamboo types.

10.3.4 AFLP

Another molecular marker technique used in bamboo identification is AFLP (*amplified fragment length polymorphism*). This technique is an RFLP combined with PCR. AFLP analysis allows precise comparisons between taxa to determine genetic distances and phylogenetic relationships, even between closely related taxa,

including infraspecies variation. The cluster pattern formed by AFLP has successfully revealed polyphyletic properties in the genus *Bambusa* (Loh et al. 2000). On the other hand, Marulanda et al. (2002) used AFLP for studying the genetic variation of *Guadua*. AFLP has also identified nine species of bamboo in Manipur State, Northeast India (Ghosh et al. 2012). However, this technique is quite tricky to apply, considering the high price. It must be done by skilled personnel, as it is difficult to be analyzed because it produces a lot of data and requires a long working time.

10.3.5 SSR

Simple sequence repeats (SSR) are molecular markers that are also applied to bamboo. SSR is a repeating sequence of tandem nucleotides with a length ranging from 1 to 6 nucleotides; is polymorphic, codominant, and multiple alleles; and is considered a neutral sequence. Therefore, SSR is widely used in the study of plant genetic diversity. SSR primers are designed from conserved genome regions, which enclose these tandem nucleotide sequences. The detected sequence lengths and polymorphisms reflect the variation in the number of repetitions between the genomes.However, all procedures that include genomic construction and screening before primer design are considered impractical and expensive (Das et al. 2008).

This greatly limits the SSR method's application to nonagricultural plants such as bamboo because sufficient genomic information is not yet available in the database. Nayak and Rout (2005) have successfully applied the use of SSR molecular markers to *Bambusa*. Six SSR sequences were isolated from *B. arundinacea* and tested on 18 other bamboo species. Three polymorphic loci are known to identify and characterize bamboo species. These findings suggest that primers designed from the *B. arundinacea* genome could be used to identify other bamboo taxa. Thus, SSR molecular markers can be used to compare taxa without having to do a specific primer design for each bamboo species. This study also shows that SSR molecular markers can be used in the study of population genetics and genetic diversity in bamboo.

10.3.6 SRAP

Zhu et al. (2014) also conducted a genetic diversity study on 13 bamboo accessions in China. A total of 21 vegetative morphological characters and SRAP (sequence-related amplified polymorphism) molecular markers were used to construct the dendrogram. In this study, the similarity coefficient obtained was used to measure genetic diversity. The similarity coefficient of 0.23–0.96 indicates high genetic diversity based on morphological characters. Likewise, the similarity coefficient of 0.36–0.75 shows high genetic diversity in molecular characters compared with

research on genetic diversity using molecular markers conducted by previous researchers.

10.4 DNA Sequence-Based Methods

10.4.1 Organelle Genes

The sequence of organelle genes began to develop since the discovery of the *rbcL* gene, which encodes the large subunit protein *ribulose 1,5 biphosphate carboxylase/oxygenase* (*rbcL*). Using this *rbcL* gene, Barker et al. (1995) describe the position of Bambusoideae among other subfamilies. However, according to Doebley et al. (1990), the *rbcL* gene's use is only suitable for taxa familia and taxa higher than family, not ideal for grasses in subfamily taxa lower than subfamily. Gaut et al. (1997) added that the woody bamboo (Bambuseae) group generally has a long generation time to slow down the rate of nucleotide substitution. It thus becomes unsuitable for an inferior taxonomic analysis. Furthermore, several chloroplast genes were found which were also used to construct molecular relationships in Poaceae, including *ribosomal protein S4* (*rps4*) (Nadot et al. 1994), *NADH-plastoquinone oxidoreductase subunit 5* (*ndhF*) (Clark et al. 1995), *maturase K* (*matK*) (Hilu et al. 1999), and *RNA polymerase b subunit* (*rpoC2*) (Barker et al. 1999).

10.4.2 Nuclear Genes

Sequencing methods with genes from the nucleus use 18S rDNA (Hamby and Zimmer 1988), *granule-bound starch synthase gene* (*GBSSI*) (Mason-Gamer et al. 1998), internal transcribed spacers (ITS) (Hsiao et al. 1999), and phytochrome B (Mathews et al. 2000). Das et al. (2008) argue that ITS is the most popular method to determine phylogenetic relationships at the genus taxon level down because it has a higher rate of nitrogen base substitution than other genetic materials.The ITS sequence data has been used to trace the phylogenetic relationships of *Thamnocalamus* and its close relative species. This sequence shows that members of the *Thamnocalamus* are monophyletic to one another (Guo et al. 2002). ITS sequences have also been used to study 23 alpine bamboo species' genetic diversity from the genus *Thamnocalamus*, *Fargesia*, and *Yushania*. The results of these studies determined *T. spathiflorus* var. crassinodus and *F. spathacea* as alpine bamboo ancestors, although these data are not supported by a useful bootstrap (Guo et al. 2002). ITS sequences have also been used for phylogenetic analysis of 21 species of *Bambusa* (sensu stricto), *Dendrocalamopsis*, *Dendrocalamus*, *Guadua*, *Leleba*, and *Lingnania*. This study concluded that *Bambusa* is closely related to *Dendrocalamus* (Sun et al. 2005).

ITS sequence, which is biparental, has been widely chosen for phylogenetic analysis at the taxon genus level and below because it has a high rate of nucleotide substitution compared to organelle genes. In addition, the ITS sequence also has many duplications, making it easy to amplify by targeting primary adhesions to conserved areas 18S and 26S, using universal primers (Das et al. 2008). However, the results of phylogenetic analyses using ITS sequences are often confusing (Alvarez and Wendel 2003). This can be due to limited information by short sequences (Baldwin et al. 1995) or difficult alignment due to varying sequence lengths (Hsiao et al. 1999).

One of the important prerequisites for phylogenetic studies using ITS sequences is targeting the correct orthologous sequence. However, in the absence of complete homogenization, paralog sequences may appear accidental and bias the results. In Bambuseae, the potential for paralog sequences is very vulnerable due to polyploidization. Another confounding phenomenon discussed by Alvarez and Wendel (2003) is the presence of a large number of rDNA copies and possible contamination due to the use of universal primers. From a number of these problems, contamination is considered a factor that affects the diversity of ITS sequence results. The genetic material (rDNA) of fungi can be accidentally isolated and amplified with the target DNA (Zhang et al. 1997). Epiphyllous fungi are known to be associated with bamboo leaves. Therefore, before DNA isolation, fresh leaves should always be sterilized first, to avoid possible contamination. Besides, researchers should not rely on the results of a single PCR reaction but attempt to be able to clone and amplify DNA products under various reaction conditions (Alvarez and Wendel 2003) to avoid PCR bias or drift (Wagner et al. 1994).

10.5 Bamboo and Molecular Descriptors

New molecular approaches have become an important aspect of the research involving in area of understanding the phenomena of genetic diversity and phylogenetic relationship. As discussed above, the new molecular techniques such as RAPD, SSR, AFLP, and RAPD are actually a trend in determining the genetic pool or genetic population of a particular plant species. Moreover, it is important to understand that all markers are not having the same characteristics and similar functions but rather they are very dissimilar in both characteristically and functionally (Karp and Edwards 1995). These molecular approaches have helped us to generate a data that has significantly helped us to find the exact roots of taxonomic complexities that could probably allow us to deal with plants that are yet to be placed in different classifications (Das et al. 2008).

Till 2000, the study of genetic diversity in bamboo was limited (Loh et al. 2000). However, the previous work done by scientists acted as a source of an encouragement to lay the hands on a large pool of genetic studies in bamboo species. Friar and Kochert (1994) conducted RFLP-based research in *Phyllostachys*. Heng et al. (1996) conducted isozyme-based studies among five genera of bamboo. Similarly,

Kobayashi (1997) conducted research on bamboos belonging to different regions of the world, and Watanable et al. (1994) conducted research based on chloroplast DNA phylogeny of bamboos belonging to Asia. A specific intron sequence of rpl16 was analyzed within *Chusquea* genus (Loh et al. 2000).

As mentioned above, RFLP technique was employed to conduct the research aimed at understanding the genetic evolution of more than 15 species of *Phyllostachys* (Friar and Kochert 1994). But this technique has shown low polymorphism as compared to others. Similarly, RAPD has provided a good alternative, as it does not need any previous information regarding the plant genome. This feature, coupled with easy accessibility in the market, has made it a good choice in studying the genetic variations among various species (Belaj et al. 2001; Deshwall et al. 2005; Ko et al. 1998). It requires very small amount of genomic DNA and can produce very high level of polymorphism and can be effective for diversity analysis in plants (Williams et al. 1993). RAPD analysis has proved its significance for diverse study of field crops like rice (Qian et al. 2001; Rabbani et al. 2008; Pervaiz et al. 2010) and many horticultural plants such as coffee (Orozco-Castillo et al. 1994), tea (Wachira et al. 1995), almond (Shiran et al. 2007), sesame (Akbar et al. 2011), and turmeric (Singh et al. 2012). Recently, large number of scientists employed molecular markers to conduct the characterization and phylogenetic relationship on bamboos (Nayak et al. 2003; Das et al. 2005; Bhattacharya et al. 2006; Ramanayake et al. 2007; Das et al. 2007; Bhattacharya et al. 2009). Moreover, SSR primers derived from rice, sugarcane, etc. were used for the study of genetic diversity among large species of bamboo (Sharma et al. 2009).

Economically bamboo plants are very important because of its multipurpose usage across the globe. China and India are the largest producers of bamboo in Asia. More importantly, bamboo is a genetically diverse plant, so it could serve as a good case of study for the better improvement and production of highly desired plant. Therefore, identification, characterization, and documentation at molecular level of the bamboo plants are essential demands in order to strategize the conservation methods of the bamboo and to improve our understanding about the taxonomy of the plant (Rao and Hodgkin 2002). Loh et al. (2000) explained that the need has arisen to collect different samples of bamboo in order to conserve the plant from further exploitation. Das et al. (2008) further explained that the molecular data of the plant can really help us to classify the plants taxonomically. Moreover, in order to assess the level of interspecies and intraspecies genetic diversity between bamboo plants, the molecular markers are considered to be a hopeful technical asset (Nayak et al. 2003). Molecular marker such as RAPD has been quite useful in revealing some important information regarding the genetic variation existing among various bamboo species. Sun et al. (2006) reported that RAPD markers were quite useful in revealing the genetic relationship between various bamboo species of the southeastern China.

Recent research is conducted by Rong et al. (2020) in which 28 species/varieties of *Bambusa* were subjected to evaluation based on 16 RAPD primers. Amplification of 216 bands were conducted by using 16 RAPD primers, which yielded about 290–3000 bp DNA fragments. It was reported that the percentage of polymorphism

were 96.79% and the number of bands was 211 which indicates that the genetic diversity (interspecific and intraspecific) was high among bamboo species. Therefore, the results suggest that the RAPD molecular markers have a practical role in detecting the variation among various species. The reason behind such a higher percentage of polymorphism could be the factors like climate variations, evolutionary changes, and geographic location that eventually set a larger genetic pool of the species (Lou et al. 2011). Nayak et al. (2003) has achieved similar kind of results; however, the number of primers he used while studying the case was higher than the aforementioned work conducted by Rong et al. (2020). It's very significant to realize that each molecular marker has an exclusive property so it would logically display different aspect of diversity of gene (Karp and Edwards 1995).

It is very tough to identify the genetic relationship between various bamboo species because of the absence of phenotypic variance. However, the confirmation of the genotype is essential for both propagators and consumers in order to protect the IPR. RAPD and ISSR markers were employed in order to evaluate the diversity of 13 genotypes of bamboos. A total of 120 RAPD and 63 ISSR primers were tried, among which only 42 polymorphic primers, 30 RAPD, and 12 ISSR reported to have produced amplification profiles. It was reported that 30 RAPD primers generated a total of 645 amplified fragments, among which about 623 were polymorphic and 20.76 polymorphic bands on each primer were detected across 13 genotypes. 12 ISSR primers generate 246 amplified fragments, of which 241 were polymorphic, and 20.08 polymorphic bands per primer were observed across 13 different genotypes. These results indicate that an extensive genetic diversity occurred among 13 genotypes of bamboo. It's very surprising that some top researchers have deemed that RAPD markers illustrate mostly noncoding regions of DNA (Bachmann 1997; Landergott et al. 2001), while some have a very different opinion and consider that RAPDs disseminate throughout the genome and link with only functional loci (Penner 1996).

Another molecular marker that we haven't discussed yet is microsatellite or sometimes also called simple sequence repeats (SSR) which have been proved to be very efficient in revealing the knowledge of genetic polymorphism among various case studies. They have very significant role in genome mapping, population genetic analysis, and genetic diversity and obviously evolutionary study (Brondani et al. 2002; Sharopova et al. 2002; Deutech et al. 2002; Kikuchi and Isagi 2002). Among bamboos, microsatellites have been characterized and identified in bamboo (*Bambusa arundinacea*) (Nayak and Rout 2005), and reportedly three polymorphic sequences have been identified in this plant which could serve as a parameter to study the population genetics among the clones of this plant and other relevant species as well.

10.6 Conclusion and Future Prospects

The use of various taxonomic evidence that includes molecular, morphological, and anatomical will provide answers to population genetics and the taxonomic status of bamboo. Research using various taxonomic evidence can produce information on genetic diversity and population structure, clarity of taxonomic identity, and bamboo relationship. It is hoped that this series of data will become the basis for tracing the evolutionary history of bamboo. Hence, the results of taxonomic identification can become a reference in a bamboo conservation strategy.

Conflict of Interest

No.

Author Contribution Author AL conceived the idea and wrote the manuscript. Author IBG, ZA, and AS reviewed the manuscript and revised the MS as per the requirements. All authors read and approved the manuscript.

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