Use of molecular marker methods in the classification of bamboo taxa: A review

Chan Nyein Khin[1]* – Anikó Veres[1] – András Neményi[2]

[1]The Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences (MATE)
[2]Institute of Landscape Architecture, Urban Planning and Garden Art, Hungarian University of Agriculture and Life Sciences (MATE)
*Correspondence: channyeinkhin1@gmail.com

SUMMARY

Bamboo plants are currently attractive to researchers because of their versatile uses. Understanding the bamboos' genetic level is needed to develop new varieties. Taxonomic identification is the basis for plant development. Bamboos were identified as their taxonomical morphological characters which are dependent on environmental factors. Molecular Marker techniques can be used to perform accurate genotype identification, which can be used for genetic diversity analyses. The RFLP, RAPD, AFLP, SSR, ISSR, iPBS, SCARS, SCoT, SRAP marker systems have been shown to be able to efficiently determine the genetic diversity of bamboo species based on genotyping. This paper summarizes research that aims to analyze the genetic diversity of bamboo species on a molecular basis.

Keywords: bamboo, genetic diversity, molecular markers, RFLP/ RAPD/ AFLP/ SSR/ ISSR/ iPBS/ SCARS/ SCoT/ SRAP markers

INTRODUCTION

Bamboo is a perennial, evergreen plant with a rapid-growing habit and belongs to the grass family *Poaceae*. The growth rate of bamboo is incredibly fast in which the height reaches up to 40 meters during 4 months after shooting (Viet Ha Tran, 2010). Its geographical distribution is a wide range: America, Africa, Asia, and Pacific regions (Fu et al., 2000) although the growth depends on the environmental factors (Sonboon, 2001). Most of the bamboos are from tropical regions, but they can be found in the temperate region too (Yeasmine et al., 2015).

Bamboo, currently, is becoming a popular trend globally because of its uses in the basic needs of humankind. Bamboo can be used for every kind of human need such as shelter, cloth, and food. Bamboo is recognized as an excellent substitute for wood in producing pulp, paper, board, handicrafts, and charcoal as well as serving mankind to provide food such as bamboo shoots and shelter and feed for animals such as cattle (Barkley et al., 2015). Moreover, it can be a solution for the environmental problems caused by erosion due to its root system (Rao, 1995). In addition, forest decline is gradually increasing so that alternative natural resources need to be found for environmental improvement. Bamboo can be considered as a solution for forest deforestation (Rao, 1995) and as a great potential of high carbon sequestration capacity (Nath et al., 2015) because of its fast-growing, rapid biomass accumulation, strong and persistent nature. Moreover, bamboos can reduce soil degradation. Proyuth et al. (2012) studied that the shift in cultivated land from annual crops to bamboos increased soil organic carbon.

Many scientists are doing research to find out the valuable properties of bamboo with different objectives. In the utilization of bamboo for people's needs, it is required to choose different suitable bamboo species with the aim of what kind of product is targeted as produce. Determining genetic diversity is one of the most important aims of research activities, and should proceed the research to find suitable plant species or varieties in order to know the genetic diversity and morphological diversity of the chosen taxa. Using molecular marker techniques is an efficient way to reliability determine genetic diversity for different research activities. This technique is the most reliable to establish the diversity of the plant taxa and it is also not too time-consuming.

IMPORTANCE OF DIVERSITY IN BAMBOO

In the *Poaceae* family, bamboo belongs to the *Bambusoideae* subfamily which includes a large number of wood bamboos. According to cytological studies, there are two distinct sections, tropical (hexaploids, $2n = 6x = 72$) and temperate (tetraploids, $2n = 4x = 48$) within the woody group. The genus *Phyllostachys* has 48 chromosomes (Das et al., 2008). Globally, there are 1,662 bamboo species, while the bamboo plantations were estimated to cover 35 million hectares around the world (FAO, 2020).

Nowadays, there is an interest in bamboos to analyze their growth performance, morphogenesis, taxonomy, distribution, ecology, reproduction, and diversity (Thakur et al., 2016). Because of their high rate of utilization, proper taxonomical identifications of bamboo taxa are highly essential (Das et al., 2008). Evaluation of the phylogenetic relationship and genetic diversity of plants is important for identifying the germplasm resources and determining the hybridization, selection procedures, and conservation. Although many species of Bamboos have been identified, most classifications depend on the morphological characteristics. However, some morphological characters in part are dependent on the environmental conditions. There is limited scientific research result available on the analysis of genetic diversity in bamboos. Therefore, more research of bamboo taxa is required to fully understand the morphological, phylogenetic relationships, and genetic diversity of the given taxa. In addition, DNA-based molecular markers can give a fast and reliable

evaluation of genetic diversity and distance (Thakur et al., 2016).

APPROACH TO THE CLASSIFICATION OF BAMBOOS WITH MOLECULAR MARKERS

There are different ways to analyze genetic diversity in plants; Morphological markers, Biochemical markers, and Molecular markers. Morphological marker means the identification of shape, size, texture, and color which are visible as physical characteristics of plants and it can be various parts of the plant (Chesnokov et al., 2020). The biochemical marker includes protein profiles and isozymes and they can be used in the genetic diversity of plants (Mahmoud and Abd El-Fatah, 2020). Molecular markers have two types; non PCR-based

marker and PCR-based marker. RFLP marker is one of the non PCR-based marker systems and RFLP, RAPD, SSR, AFLP, etc. are involved in PCR-based markers (Kumar et al., 2018). Molecular marker techniques based on genotyping of individuals can be used effectively in the analysis of genetic diversity. By using these molecular methods, results with useful information can be obtained for the taxonomic studies of bamboo. *Table 1* summarizes the chronologically published markers and their application to bamboo genotypes. One of the main objectives of research in bamboos has been to analyze the genetic diversity of bamboos. According to *Table 1*, the researchers have been trying to unravel the genetic correlations of bamboo for many years and there is still a need to study the bamboo genetic classification.

Table 1: **The Marker systems, published year and year used in bamboo genetic diversity study**

Marker name	Published year	Reference	The year that started to use in the bamboo study	Reference
RFLP	1980	Botstein et al., 1980	**1991**	Friar and Kochert, 1991
SSR	1989	Litt and Lutty, 1989	**1997**	Lai and Hsiao, 1997
RAPD	1990	Williams et al., 1990	**1997**	Gielis et al., 1997
SCARs	1993	Paran and Michelmore, 1993	**2005**	Das et al., 2005
ISSR	1994	Zietkiewica et al., 1994	**2009**	Lin et al., 2009
AFLP	1995	Vos et al., 1995	**2000**	Loh et al., 2000
SRAP	2001	Li and Quiros, 2001	**2014**	Zhu et al., 2014
iPBS	2004	Schulman, Flavell, Ellis, 2004	**2019**	Li et al., 2019
SCoT	2009	Collard and Mackill, 2009	**2020**	Amom et al., 2020

Restriction fragment length polymorphism (RFLP) determines polymorphisms for the genotypes under study based on digestion of the genome by restriction enzymes and hybridization assays (Botstein et al., 1980). RFLP is also used to discriminate genetic variability in bamboo. Friar and Kochert (1991) used an RFLP marker system for 61 bamboo samples and genotyped 21 species belonging to 2 genera (Friar and Kochert, 1994). They concluded that RFLP is effective for detecting genetic polymorphism within and between bamboo species.

The technique of **microsatellite or simple sequence repeat (SSR)** markers is very well known to discriminate genetic diversity and is used in a variety of plants. SSR markers are short tandem repeat sequences (1-6 nucleotides in length) and primers are designed from conserved genomic regions accompanying the repeat sequences (Malay Das et al., 2008). SSR is widely used for genetic diversity classification and genetic mapping to detect important traits in plants. This marker technique has been successfully applied to *Phyllostachys* bamboo species since 1997 (Lai and Hsiao, 1997). Nayak and Rout (2005) studied 18 bamboo species under the genera, *Bambusa, Dendrocalamus, Dinochela, Cephalostachyum, Bambusa, Sasa,* Shibata, *Arundinaria*, by using 6 SSR markers developed from *Bambusa arundinacea* which

were 3 monomorphic and 3 polymorphic characters and they concluded that 3 polymorphic microsatellite loci can be used. Meena et al. (2019) used the 17 SSR primers pairs developed from *D. latiflorus* and *Bambusa arundinacea* to study genetic diversity analysis of 19 natural stands of *Dendrocalamus hamiltonii* which is a commercial bamboo species in the Northeast Himalayas. Sharma et al. (2008) observed the genetic diversity of 43 bamboo accessions of 23 bamboo species belonging to *Dendrocalamus, Bambusa, Phyllostachys, Ochlandra, Sasa* and *Melocanna* genera with 42 SSR markers (34 rice SSR out of 98 and 8 sugarcane SSR out of 20) revealed the 73% variation among bamboo species. The study showed these markers determined high diversity between species, however, low diversity level within species. 54 EST-SSR (expressed sequence tag-simple sequence repeats) have been used to study genetically the genotypes of 16 Lei bamboo (*Phyllostachys violascens*) varieties and other 9 different *Phyllostachys* species; *P. glabrata, P. verrucosa, P. bambusoides, P.aurea, P. edulis, P. virella, P. rivalis, P. parvifolia,* and *P. Nidularia* and observed to be well suited for genetic segregation within *P. violascens* varieties and between *Phyllostachys* species (Cai et al., 2019). Disasa et al. (2016) genotyped 9 bamboo species, *Gigantochloa apus, G. atter, G. Sumatra,*

Guadua angostifolia, G. amplexifolia, Bambsa testiles, Arundinaria alpina, Oxytenanthera abyssinica, and *Phyllostachys bambusoids*, using 90 SSR markers developed from sorghum and 15 among 90 sorghum microsatellites markers showed polymorphic amplification and were highly transferrable to all tested bamboo species, suggesting that these 15 markers can be used to distinguish among bamboo species. 120 rice SSR markers were used under investigation of 21 different bamboo species under the genera of *Arthrostylidium, Arundinaria, Bambusa, Dendrocalamus, Melocarna, Phyllostachys, Pleioblastus, Pseudosasa, Sinobambusa* and *Thyrsostachys* for their diversity, and 31 out of 120 markers showed the polymorphism among tested bamboo species (Chen et al., 2010). Thirteen microsatellites developed from the genotypes of bamboo species, *Aulonemia aristulata*, were used to investigate genetic variability of 18 different bamboo species, *Bambusa beecheyana, B.longispiculata, B. malingensis B. oldhamii, B. stenostachya, B. textilis, B. tulda, B. tuldoides, B. ventricose, B. vulgaris cv vittate, B. vulgaris, Dendrocalamus asper, D. giganteus, D. latifl orus, D. strictus, Gigantochloa apus, G. verticillate, Guadua amplexifolia* and seven out of thirteen showed as useful primers for bamboo species (Abreu et al., 2011).

Randomly Amplified Polymorphism DNA (RAPD) is the method that uses the short length primer (short oligonucleotides (10 base pairs)). The primer can bind to the complementary sequences in the DNA of the tested samples, then it can amplify and visualize the amplified DNA. This marker system was developed in 1990 (Williams et al., 1990) and used in bamboos since 1997 (Gielis et al., 1997b as cited in Das et al., 2008). This method has some advantages which are low cost, fast technique, and information of plant genome are not needed as well as being the most popular technique. It can also determine the relationship between varieties and between species.

According to the advantages described above, several research analyses carried out the genetic diversity of bamboo by using this marker technique. Nayak et al. (2003) used 10 RAPD primers in 12 species of bamboo namely, *Bambusa vulgaris, B. vulgaris* var. striata, *B. ventricosa, B. multiplex* var. Silver stripe, *B. multiplex, B. arundinacea, B. balcooa, Dendrocalamus giganteus, D. strictus,* Dinocloa *m'Clellandi, Cephalostachyum pergracil* Munro, and *Sasa* species and 137 distinct polymorphic DNA fragments were amplified among the species studied. Nath et al. (2013) found the RAPD markers gave a high level of polymorphism in genetic diversity within 28 accessions of reed bamboo (*Ochlandra travancorica*). Shalini et al. (2013) found that 7 out of 21 RAPD markers had high polymorphisms that discriminated between 10 bamboo genotypes, *Bambusa tulda, B. nutans, B. balcoa, B. vulgaris, B. bamboos, Dendroclamus strictus, D. giganteus, D. asper,* and *Guadia angustifolia*. Desai et al. (2015) used RAPD markers to differentiate 13 Indian bamboo genotypes and found more than 60% polymorphism used by

RAPD markers. Tiwari et al. (2019) used 10 *Thamnocalamus spathiflorus* genotypes in a RAPD based genetic diversity study, in which they found that RAPD is a useful marker for estimating close or distant relationships between species and bamboo groups. In Indonesia, 25 species from five different bamboo genera which were *Schizostachyum, Bambusa, Gigantochloa, Dinochloa,* and *Dendrocalamus* have screened with 40 RAPD markers and 24 of 40 primers accounted for 86.21% of polymorphic bands (Annisa et al., 2019). Tamilmani Eevera et al. (2008) studied 10 RAPD markers in *Bambusa* and *Dendrocalamus* genotypes. Their results showed that *D. brandisii* was clustered alongside *Bambusa,* in a cluster, while *D. giganteus* was very distant from the other *Bambusa.* Therefore, it can be assumed that the RAPD marker technique is quite useful to distinguish the genetic diversification of bamboo taxa. It can give a satisfactory and reliable result related to genetic diversity between species as well as within species. However, some studies have shown that the RAPD marker is not able to amplify in all samples. Annisa et al. (2019) used 40 RAPD markers to assess the genetic diversity of bamboo, but only 24 primers proved useful. Likewise, Shalini et al. (2013) observed that 7 out of 21 RAPD markers were able to discriminate bamboo genotypes, implying that 30% of the RAPD markers used are reliable for detecting genotypes for diversity. Although only 30–40% of the primers used can give positive polymorphisms, Sawarkar et al. (2021) suggested that it is the most popular because the technique is fast, cheap, and easy to use.

In addition, the RAPD developed from the technique RAPD-RFLP, which involves random amplification (PCR) followed by restriction enzyme digestion and gel electrophoresis (Konzen et al., 2017). Genotypes of genera and species belonging to 13 bamboo taxa, which are: *Bambusa vulgaris vittate, B. vulgaris, B. beecheyana, Phyllostachys edulis, P. heterocycle, Dendrocalamus asper, D. giganteus, Guadua amplexifolia, Guadua superba,* and other 4 unidentified species of *Guadua* were used in diversity analysis using the RAPD-RFLP method, the digestion of RAPD products from the primer OPV-17 with the three enzyme combinations (*MspI, HindIII/HaeIII,* and *HinfI/RsaI*), and it gave 79% higher polymorphic bands compared with 12 RAPD primers (Konzen et al., 2017).

Sequence-characterized amplified region (SCAR) markers are sequence-requiring, locus-specific, and PCR-based markers. They were first developed from RAPD markers, which research was linked to lettuce disease resistance genes (Paran and Michelmore, 1993). Das and colleagues (2005) were the first to report two bamboo-specific SCAR primer pairs applied to 15 bamboo species. In their study, they identified species-specific SCAR markers for *Bambusa balcooa* and *Bambusa tulda* named the Balco836 marker for *B. balcooa* and the Tuldo609 marker for *B. tulda*, which were derived from polymorphic fragments of RAPD markers. Rangsiruji et al. (2018) used 5 SCAR primers designed for the polymorphic RAPD

fragments in bamboo taxa. Subsequently, polymorphisms were observed among 8 *Dendrocalamus* bamboo taxa and these primers were able to discriminate between individuals of the bamboo taxa under study.

Inter Simple Sequence Repeat (ISSR) markers, nowadays, are widely used in genetic diversity investigation in plant species such as *Sorghum bicolor* and banana (Godwin et al., 1997), Lily (*Lilium* L.) species (Žukauskienė et al., 2014), *Jasminum* L. (Akhtar et al., 2021), *Ziziphus spina-christi* (L.) Willd (Alansi et al., 2016), *Cycas guizhouensis* (Xiao et al., 2004) and desert date (*Balanites aegyptiaca* Del. (Abdelaziz et al., 2020). ISSR markers have also been shown to be useful in classifying bamboo species. In the case of bamboo species, 93 individuals of *Melocanna baccifera* from 7 locations, which are commercially important bamboo in North-East India, were assessed for population genetic diversity using 5 different ISSR markers and the result showed significant levels of genetic variation within the populations (88.37% of polymorphic bands) (Nilkanta et al., 2017). Twelve ISSR primers and four expressed sequence tag (EST)-based random primers were used in twenty-two accessions belonging to 9 species of *Bambusa*, 5 species of *Dendrocalamus*, 2 species of *Pleioblastus*, 1 species of *Melocanna*, *Oxytenanthera*, *Phyllostachys*, *Thyrsostachys*, *Schizostachyum*, *Sasa*, and 220 polymorphic bands were amplified (Mukherjee et al., 2010). Amom and colleagues (2018) observed 15 different bamboos, *Bambusa tulda*, *B. nutan*, *B. mizorameana*, *B. vulgaris*, *B. manipureana*, *Schizotachyum dullooa*, *S. pergracile*, *S. munroi*, *S. fuchsianum*, *Dendrocalamus giganteus*, *D. hamiltonii*, *D. sikkimensis*, *D. hookeri*, *D. longispathus* and *D. manipureanus* from North-East India to study the phylogenetic relationship between species by using 10 ISSR markers. They observed the genetic variation among those species and found those species belonged to two cluster groups. Yang et al. (2012) analyzed 12 accessions of the woody bamboo (*Dendrocalamus membranaceus*) from Yunan Province by using 10 ISSR markers. Based on their result, 98.71% of the loci examined were found to be polymorphic.

The Amplified Fragment Length Polymorphism (AFLP) method was published in 1995 by Vos and colleagues using genotypes of Arabidopsis thaliana, tomato (*Lycopersicon esculentum*), and maize (*Zea mays*) species. The AFLP method involves cleavage of genomic DNA molecules with restriction enzymes, subsequent ligation of adaptor molecules to unknown genomic fragments, and PCR amplification followed by detection of polymorphisms by electrophoresis. In Japan, Obayashi and Hayashi (2000) screened 22 dwarf bamboos (*Sasa senanensis*) with 3 AFLP primer combinations and concluded that the AFLP primer they chose is suitable and reliable for the clonal discrimination. Ma Q-q and colleagues (2013) used 10 AFLP primers to investigate the genetic diversity of dwarf bamboo (*Bashania fangiana*) populations of two different genetic ages in China. Genetic polymorphism and relationships were detected in 15 bamboo species

of 4 genera, *Bumbasa*, *Gigantochloa*, *Dendrocalamus*, and *Thyrsostachys* belonging to subtribe *Bambusinae* with 8 AFLP primer combinations by Loh et al. (2000). The fifteen bamboo taxa including ten *Dendrocalamus* and each species of five different bamboo genera, *Bambusa*, *Dinochloa*, *Melocalamus*, *Oxytenanthera*, and *Thyrsostachys* were tested with 5 AFLP primer combinations and it was concluded that AFLP assay detected polymorphism (99.2%) in 15 bamboos species (Pattanaik and Hall, 2011). Johan Gielis et al. (2004) used AFLP methods to 47 genotypes belonging to 5 different bamboo taxa which are *Phyllostachys edulis*, *P. aurea Koi*, *Bambusa ventricose*, *B. vulgaris*, *Sasa veitchii*, and the results showed that 39 genotypes were clearly distinguishable, making the AFLP method suitable for genetic diversity studies. The AFLP analysis was found to be suitable for testing the genetic distance between the bamboo species under study, using 18 different landraces of bamboo from nine species of four genera namely, *Bambusa*, *Dendrocalamus*, *Melocanna*, *Thyrostachys*, in India (Mandi et al., 2012). In addition, the AFLP method can be used to study genetic purity. Six AFLP primer combinations have been used in a clonal identity study of *Bambusa nutans* Wall bamboo species that were grown under *in vitro* environment, a 98.8% genetic identity was detected among them (Mehta et al., 2010). Isagi et al. (2004) confirmed that in their study, AFLP analysis was able to distinguish between two different genotypes with flowering intervals of 67 and 69 years.

Sequence- Related Amplified Polymorphism (SRAP) marker is one of the dominant marker techniques and it is simple, low cost, and effective in high reproducibility (Robarts and Wolfe, 2021). This primer targets the GC-rich exons as forward primer and AT-rich promoter, introns, and spacers as reverse primers and the length of primer are 17 or 18 nucleotides long (Li and Quiros, 2001). Robarts and Wolfe (2021) pointed out that the SRAP marker technique can be used in specific research areas of plant biology such as hybridization, conservation genetics, and ecology. Zhu et al. (2014) also applied SRAP marker for the first time in bamboo genetic identification. 13 bamboo accessions belonging to 5 different genera namely, *Bambusa*, *Phyllostachys*, *Pleioblastus*, *Shibataea*, subgen. *Pseudosasa* were tested with 86 SRAP primer combinations, and 38 out of 86 primer pairs showed 80.65% polymorphism. According to the result, they concluded that the SRAP technique can be used as an efficient method in assessing genetic diversity within bamboo genotypes.

Inter- Primer Binding Site (iPBS) is a random technique based on primers designed for the LTR region of the LTR retrotransposon (Kalendar et al., 2018). Amom et al. (2020) used 10 iPBS markers to assess genetic diversity in 5 native bamboos genotypes (*Bambusa cacharensis*, *B. mizorameana*, *Dendrocalamus manipureanus*, *D. hamiltonii*, and *D. sikkimensis*). The results showed that these primers are able to detect polymorphisms between the bamboo genotypes studied. Li et al. (2019) observed the genetic diversity and population structure of 58 Asian bamboo

accessions belonging to the genus *Phyllostachys* with 16 inter-retrotransposon amplified polymorphism (IRAP) markers and these primers gave 98.3% polymorphic and 1.7% monomorphic amplicons to discriminate the diversity among Asian bamboo accessions.

In the case of **Start Codon Targeted (SCoT)** markers, the only primers include the genes translational start code (ATG) and those short-conserved sequence environment, plus 3' end-selective nucleotides (Collard and Mackill, 2009). It is a dominant marker and can be used in many plant species for genetic diversity studies, phylogenetic analysis, and marker-assisted selection (Collard and Mackill, 2009). Amom et al. (2020) compared the applicability of the RAPD, ISSR, iPBS, and SCoT markers in bamboo and concluded that the SCoT marker is the most effective of the four markers because it has the highest polymorphism information content for phylogenetic analyses.

Amom et al. (2020) claimed that RAPD, ISSR, iPBS, and SCoT markers are efficient marker techniques to study the genetic relationship between bamboos. Lin et al. (2011) also approved that ISSR, SRAP, and AFLP markers are useful methods in the identification of genetic diversity among *Phyllostachys violascens* cultivars. Sawarkar et al. (2021) reported that molecular marker techniques such as SCoT, RAPD, ISSR, iPBS, AFLP, and RFLP are being used by researchers to study the species and/or generic levels of bamboos. *Table 2* showed the list of markers and their characteristics according to Sawarker et al. (2021).

Table 2: **List of Markers and their characteristics (Sawarkar et al., 2021)**

Function	SCoT	RAPD	ISSR	iPBS	AFLP	RFLP
Prior sequence information required	No	No	No	No	No	Yes
Rapid/Speed	Fast	Fast	Fast	Fast	Slow	Rapid
Sample DNA amount	Small	Small	Small	Small	Small	Large
Reproducibility	Highly	Low	High	–	Low	High
Informative	More informative	less	Medium	Informative	More informative	informative
Reliability	More reliable	Low	Medium	Reliable	More reliable	Highly reliable
Cost	Expensive	Inexpensive	Low	Medium	Inexpensive	Expensive
Popularity	Less	Most	More	Less	More	Popular
Dominant Marker	Dominant	Dominant	Dominant	–	Dominant	Codominant
Simpler	Yes	Yes	Yes	–	No	No
Complexity	No	No	No	–	Yes	Yes
Radioactive labelling desirable	–	No	No	–	Yes	Yes
Restriction enzyme required	–	No	No	–	Yes	Yes
Gene- targeted	Yes	No	No	Yes	No	–
Primer length	Longer	Shorter	Long	–	–	Longer
DNA fingerprinting technique	Yes	Yes	Yes	Yes	Yes	Yes
Produces polymorphic fragments (bands)	High	Lowest	Low	–	High	–

Except for the RFLP marker, the other marker systems do not need to require sequence information (Sawarkar et al., 2021) so that new plant species that are not well known can be observed by those marker systems.

CONCLUSIONS

Bamboo has been recognized as a versatile plant because it can be used in construction, making of furniture, biofuel, cloth, paper production, food as well as grown as ornamental plants in gardens, parks. In addition, it can improve the environment because of its fast growth, root system, and leaves. Nowadays, people concerned about climate change, try to find ways of protection from climate change. In this case, bamboo is popular because of its growth habit and root system. The bamboos can absorb CO_2 much more than other plants and it can reduce soil degradation. Moreover, its root system can decrease soil erosion. Many scientists in every scientific area carried out experiments related to bamboos with different aims because bamboo is known as one of the beneficial plants for humankind. Therefore, it is needed to develop superior bamboo varieties for different purposes. It is necessary to find out if there is enough genetic diversity within bamboo taxa prior to producing superior varieties. Diversity in genetic resources can support the chances for the plant breeders in new cultivar development with desirable characters. In addition, the taxonomy of bamboos is the basic and the most important process to reach the required goals. In the past, the taxonomy was identified

from morphological characteristics, however, that identification method relies on environmental factors. They can only be visible in the developmental stages of the plants, but not recognized at the genetic level. Moreover, the numbers of morphological markers have great limitations. Scientists cannot recognize juvenile plants from only morphological characters during bamboo species identification, however, can recognize them with the help of molecular marker techniques. Nowadays, the number of molecular markers is unlimited and marker systems are developing all the time. Some reviews mentioned useful marker systems for genetic diversity analysis of bamboo taxa, however, this review shows that there are much more marker techniques that are already used in bamboo genetic diversity analysis. Therefore, it can get a wide range of knowledge about marker techniques that are being used in genetic diversity analysis for bamboo species from this review. With greater progress in molecular marker systems, other superior markers may also emerge in the future and it can speed up the bamboo plant breeding process. Marker techniques can also determine the genetic identity within the plant population. As bamboos' flowering time does not occur every year after mature growth, it is very difficult to get seeds from mother plants. The bamboo plant population relies on their vegetative parts, especially from rhizomes. The genetic identity in clonal structure within the relevant bamboo population can be determined with molecular techniques, for example, AFLP primers were used to identify the clone structure of bamboo species and the desired result was obtained in which those primers can investigate the same genetic identity within the clones in a short time compared to morphological measurements. SRAP Marker can also distinguish different clones from each other, even the divergent clones. Nowadays, environmental changes lead to the extinction of some species including plant species. Bamboo species are also declining in nature because of overutilization. Molecular marker systems can characterize the endangered genotypes and can help to identify and conserve these taxa for protection. It had been shown that marker combinations such as RAPD-RFLP combination gave the highest polymorphism compared to RAPD marker technique in bamboo genetic characterization. However, very little research that used this marker combination method in bamboos can be found so that it is needed to continue the investigation with this method for genetic diversity analysis in bamboos. In brief, molecular marker systems can provide the understanding of bamboos genetic diversity and it can lead to improving bamboo production with the desirable characteristics.

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