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ROLE OF MOLECULAR MARKERS TO STUDY GENETIC DIVERSITY IN BAMBOO: A REVIEW

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Review Article

ABSTRACT

The genus *Bambusa* (family Poaceae) is the fastest growing grass in the world and adapted to various climatic conditions. Bamboos are used for variety of purposes such as for making poles, paper, charcoal, candles. Most of the species of the bamboos are edible and have high nutritional and mineral value. Bamboo shoots, leaves and other parts are used as food both in the fresh and dried form. Edible bamboos are used as tea, pickles and many more due to their high nutritional value. The flowering in bamboo is infrequent and seed viability is low, to fulfil the requirements of the bamboo *In vitro* culture of bamboo can be done. Different explants are used to grow the bamboo in the laboratory. It is present all over the world with a great genetic diversity and to classify the bamboo traditional methods are not appropriate. There are around 1200 species indigenous to Asia and new world of bamboo and their genetic diversity can be evaluated by using different molecular markers. A wide range of molecular markers is available that can help in the classification of bamboo and identification of bamboo genetic diversity. The present review highlights the use of molecular markers that are used in the genetic evaluation of the bamboo species.

Keywords: *Bambusa*; *Drepanostachyum falcatum*; alkaline soil.

INTRODUCTION

Bamboo (*Bambusa*) is a perennial grass that grows in diverse climate and soil rich in moisture. Bamboo grows in all areas except alkaline soil, deserts and marshy areas [1,2]. There are 60-70 genera containing 1200 to 1500 species in the world. Bamboo is divided into two parts - rhizome, it is an underground parts and culms that is hollow and cylindrical and grow fullest in 3 to 4 months [3]. Family Poaceae contain 12 sub-families which include Bambusoideae as one of the sub-family containing bamboo species. According to Liese et al. [4] molecular sequence study of bamboo is originated from different lineage. Bamboo is fastest growing grass and a great source of non-timber. It contains chemical compounds like flavonoids, phenolic and essential oils [5]. Some species of bamboos are edible in nature that can be consumed in different forms like dried, fresh or fermented. Bamboo leaves are used as a healthy drink in many parts of the world.

Bamboo tea does not contain caffeine and is a rich source of proteins and minerals [6]. In Asian medicine, bamboo plays an important role For example bamboo leaf extracts, shoots of bamboo are used as an anti-oxidants, anti-microbial whereas there toxic effects leads to male sterility and have negative effects on the thyroid. Sometimes leads to cardiovascular diseases also [7]. *Drepanostachyum falcatum* is known as Himalayan weeping bamboo in Uttrakhand and used in making baskets, mats, and pots. Because of overexploitation of bamboo species and less availability of seeds, new methods are required because conventional methods is insufficient to meet the demand. So tissue culture is adopted as a rapid source for bamboo propagation [8]. For the conservation of the bamboo, it is important to analyse their genetic diversity. Genetic makeup of an organism helps in the adaptability in the environment. Different molecular markers have been used to analyse the genetic diversity of the bamboo [9]. Inter Simple Sequence Repeats (ISSR) markers are widely used for the study of genetic variability than the RAPD markers [10]. Randomly Amplified Polymorphic DNA (RAPD) is simple and rapid method but have sensitivity to the reaction condition. ISSR markers are used to determine intra-genomics and inter-genomics diversity [9].

BAMBOO- A GOLDEN GRASS

The bamboo plant plays an important role in the ecosystem [11]. It has fast growth and have high nutritional value. Bamboo shoots and leaves are used for various purposes [12]. In Manipur (India), almost all the bamboo species that are grown are used for edible purpose. Their shoots are soft and tasty. Five popular species i.e *Bambusa nutans, B. tulda, Dendrocalamus giganteus, D. hamiltonii and D. sikkimensis* were taken to study the nutritional value of the bamboo containing the macronutrient value and mineral value revealing that out of five species three species (*B. nutans, D. giganteus and D. hamiltonii*) have high nutritional value and have high yield also [13]. Bamboo shoots are rich in bioactive compounds and have various positive effects on the body, like reducing cholesterol etc. Bamboo shoot can be used for edible purposes but due to their seasonal unavailability, shoot powder can be used. According to Choudhury, Monisha, et al. [14] *Bambusa balcoa* shoot were taken and used in biscuit making. Different concentration of shoot powder were taken (0%, 5%, 10%, 15%) replacing the wheat flour. Their different Physico-chemical properties were observed concluding that up to 10 % bamboo shoot powder can be used in biscuit making. However, various diseases are known that affect the growth and production of bamboo. These diseases are due to viruses, fungi, bacteria and phytoplasma. By proper management of the diseases, the production and growth of the bamboo can be increased [15]. Large number of plantlets can be produced by micro propagation and cryopreservation of somatic embryos is done to store the germplasm for future use [16,17].

TISSUE CULTURE OF BAMBOO

Plant tissue culture based techniques allow us to grow disease free plants from small explants in artificial nutrient medium under controlled conditions. Nowadays, it is widely used for the conservation of endangered plant species, production of secondary metabolites and to maintain crop germplasm repositories [18,19,20]. However, these techniques are expensive and require more labour. Moreover, unhygienic practices in plant tissue culture lab may cause culture contamination, browning of the explants etc. Tissue culture allows growing the bamboo aseptically [21]. In China, Moso bamboo a monopodial bamboo is an important forest crop. It is economically important but due to low seed viability, conventional breeding is limited. There are many tissue culture techniques for sympodial bamboo but for monopodial bamboo, it is less. Seeds of Moso bamboo are taken as an explant and by somatic embryogenesis protocol plantlets are produced from the explants [22]. As the demand for the bamboo and its products are increasing, multiplication through seeds are not sufficient to meet the demand. Waikhom [23] found an effective protocol for the micro propagation of two species *Bambusa tulda* and *M. baccifera* through nodal segment. They both are sympodial in nature and have high nutritional value. They found that 3 mg/BAP in MS media is effective for both breaking of bud and multiplication of *B.tulda* and *M. baccifera*.

APPLICATIONS OF BAMBOO

In recent years, Bamboos are using in both the engineering and non-engineering fields. Bamboo is a multipurpose plant and use in variety of things. All parts of the bamboo are used in the different fields [24]. Bamboo is used as a modern engineered material. As the demand for wood and wooden products are increasing, alternative products are explored. Bamboo provides an alternative for the wood. Traditionally, it has been used in paper and pulp industry but now bamboo charcoal and green buildings and roofs are developing [25]. Bamboo, as multipurpose grass has great potential in the field of biorefinery. It can provide a valuable feedstock in this field. Hemicellulose present in the bamboo provides different alternatives and can be a great source for biofuels, biomaterials and food [26]. As the safety issue is concern, the polymers are being used in the industries for different materials. Bamboo also provides the polymers mixed with bamboo fibres for polymer composite [27]. Bamboo is mostly used in industries but it also provides bioactive compounds and natural antioxidants. Every part of the bamboo is useful but the shoots and leaves are used in functional foods and nutraceuticals. Bamboo as grows very fast so provide a good option for natural antioxidants for the body [28]. Fig. 1 illustrate the major applications of the bamboo.

CLASSIFICATION OF BAMBOO

An extensive diversity exists in the complex bamboo germplasm. There is great need for the classification of the bamboo species to identify them. Different classification system have been proposed for the classification of the bamboo. Some scientist used morphological classification whereas some classify the species on molecular basis.

MORPHOLOGICAL CLASSIFICATION

The grass family Poaceae contains 12 sub-families. Bamboo comes under the sub-family bambusoideae. In the mesophyll of the bamboo, asymetric arm cells are present. The leaf blade of the bamboo is pseudopetiolate and have fusoid cells with vascular bundles. On the basis of region and morphological characters bamboo is classified into three categories; 1) Arundinarieae called temperate woody bamboos, 2) Bambuseae tropical woody bamboos, and 3) Olyreae herbaceous bamboos [31] and presented in the Figs. 2 and 3. As conventional methods of taxonomy is based on morphological characters and flowering, provides the main hurdle in the bamboo because the flowering is irregular in bamboo. This restrict the study of reproductive characters of the bamboo [32].

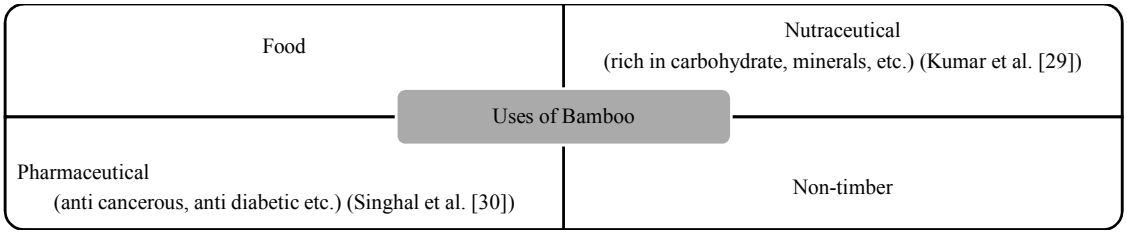

Fig. 1. Major applications of bamboo

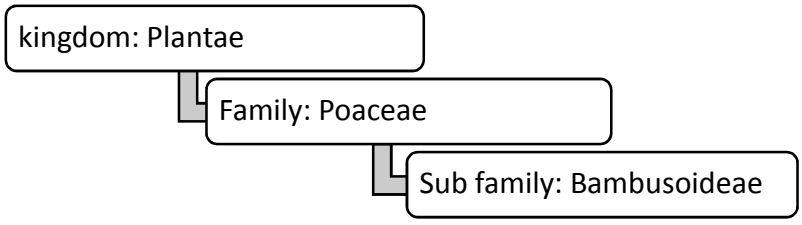

Fig. 2. Classification of bamboo [31]

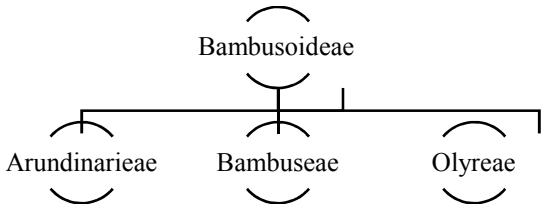

Fig. 3. Bamboo classification [31]

MOLECULAR MARKERS BASED CLASSIFICATION

Molecular Markers

To develop crops with desirable traits, genetic engineering and biotechnological techniques are very useful [33,34]. Molecular markers are used to select desirable traits in the crops [35]. Molecular markers can also be used to identify the polygenic characters which were difficult with traditional methods [36]. Along with taxonomy, molecular markers are also used in the herbal drug technology that is change of botanical material into the medicine [37]. Molecular markers have been used for different purposes. Different molecular markers are used for genomic study of various plants including bamboo [38,39,28,40, 41,10].

In vitro flowering are used for some bamboo species due to unusual flowering pattern and molecular markers are also used to classify the bamboo species as morphological features are less reliable [42]. For the conservation of bamboo, the study of its genetic variation is important. Twenty-five species of bamboo have been taken from the Indonesian Bogor Botanical Garden (BBG) and their genetic diversities were estimated using the RAPD markers. High level of polymorphism was present among the species [43]. India has a rich source of biodiversity and have many hotspots throughout the country. One of them is Western Ghats. Two species of bamboo, *Dendrocalamus strictus and Bambusa bambos* are widely distributed in the Western Ghats. Economy of India and many other countries mainly depend on the bamboo and its products. Elite clones of the two species are identified and their molecular characterization is done by using RAPD markers. Results showed that 61.40% to 84.23% similarity in the *Dendrocalamus strictus* species and 51.58% to 93.11% in *Bambusa bambos* species [44]. Traditional methods of classification have many limitations and are less reliable, therefore molecular markers are used for the characterization. 15 different species were collected from the north-east India and phylogenetic relationship can be analysed by using ISSR markers [45]. RAPD and ISSR markers were used in the genetic assessment of the 13 different species of bamboo. Total 846 polymorphic bands were produced by the both markers, dividing the 13 different genotypes in the two major clusters [46]. 13 Indian bamboo species (9 species of bambusa and 4 species of phyllostachys) are used to assess their genetic diversity. 120 RAPD and 63 ISSR primers were used among which 42 polymorphic primers were having amplified profile and are further used resulting in large genetic diversity among 13 different species [46]. From the *Bambusa oldhamii*, 10 EST-SSR markers were used identified and used to check the polymorphism in 25 bambusoideae species [32] whereas *Dendrocalamus hamiltonii* were also used to find the EST-SSR markers and also have high level of diversity [42]. A summary of all the markers used in different bamboo species are cited in the Table 1.

Types of Molecular Markers

Molecular markers play a significant role in plant biotechnology and genetic study [65]. From the last few decades molecular markers are used to detect the polymorphism in the DNA. Broadly, molecular markers are classified as Non PCR based and PCR based DNA markers [66]. Along with molecular markers, biochemical markers can also be used in plant genetic diversity identification for example in pea plants [67]. These markers are used in different fields like conservation of forest genetics resources etc [68].

Table 1. Markers for genetic analysis in different bamboo

Marker type	Marker	Genus/ Species	References
Biochemical markers	Isozyme	*Sinarundinaria anceps, Drepanostachyum falcatum, Bambusa oldhamii, Bambusa arundinacea, Dendrocalamus strictus*	Tiwari et al. [47,48], Hsieh et al. [49], Chaluvaraju et al. [50]
	Allozyme	*Raddia brasiliensis, Raddia lancifolia, Raddia megaphylla ,Raddia stolonifera, Raddia soderstromii, Bambusa bambus, Bambusa balcoa, Bambusa multiplex, Bambusa nutans, Bambusa pallida, Bambusa polymorpha, Bambusa tulda, Bambusa vulgaris, Bambusa vulgaris var. striata, Bambusa vulgaris var. wamin, Dendrocalamus asper, Dendrocalamus giganteus, Dendrocalamus hamiltoni, Dendrocalamus longispathus, Dendrocalamus membraneceous, Dendrocalamus strictus, Gigantochloa albaciliata, Gigantochloa black bamboo, Melocanna baccifera, Pseudooxytenanthera, Thyrsostachys olivera*	Oliveira et al. [51], Bhandari et [52]
Molecular marker	RFLP	*Phyllostachys nigra*, *Bambusa vulgaris Schrad.*, *B. vulgaris var. vittata Rivière*, *C. Rivière*, *B. beecheyana Munro*, *Dendrocalamus giganteus Wallich ex Munro*, *D. asper, Backer ex k. Heyne* , *Phyllostachys edulis*, *P. heterocycla (Carrière) Mitford., Guadua amplexifolia J. Presl, G. superba Huber*, *P. heterocycle*, *Guadua amplexifolia* , *G. superba Huber*, *Bambusa balcoa*, *Bambusa vulgaris*, *Bambusa pallido munro*	Friar et al. [53], Konzen et al. [41], Senet et al. [54]
	RAPD	*Bambusa vulgaris, Bambusa vulgaris var. striata, Bambusa ventricosa Maclure, Bambusa multiplex var. Silver stripe, Bambusa multiplex , Bambusa arundinacea Willd, Bambusa balcooa , Dendrocalamus giganteus Munro, Dinocloa m'Clellandi Kurz, Cephalostachyum pergracil Munro, Dendrocalamus strictus, Sasa species Makino, Bambusa tulda, Phyllostachys vivex*	Nayak et al. [55], Das et al. [56], Desai et al. [46]
	AFLP	*Trimeresurus stejnegeri (Schmidt), phyllostychus nigella, P. glabrata,, P. vivax f. Huangwenzhu, P. vivax f. aureocaulis, P. circumpilis, P. dulcis ,P. vivax f. vivax , P. yunhoensis , P. iridescens, Ochlandra travancorica*	Creer at al [57], Lou et al. [58], Nag et al. [59]
	ISSR	*Dendrocalamus membraneceous , Dendrocalamus giganteus Munro, Bambusa balcoa, Bambusa bambos, Bambusa multiplex, Bambusa vulgaris, Phyllostachys vivex, Bambusa tulda, Dendrocalamus strictus (Roxb.)*	Yang et al. [60], Tian et al. [61], Desai et al. [46], Goyal et al. [62].
	SSR	*phyllostachys glabrata, P. verrucosa, P. bambusoides, P. aurea, P. edulis, P. virella, P. rivali, P. parvifolia, P. nidularia Dendrocalamus ,Bambusa,Phyllostachys, Ochlandra, Melocanna baccifera*	Cai et al. [63], Sharma et al. [64]

Biochemical Markers

Biochemical markers are used to detect morphological changes in the embryogenic culture of *Eurycoma longifolia*. Proteins, isozymes and ethylene are different types of biochemical markers and are used in the differentiation of embryogenic tissue from non-embryogenic tissue in the plant *Eurycoma Iongifolia Jack* [69]. Different biochemical markers are used that provide information about the effect of abiotic stress in the plants [70]. Biochemical markers can be an effective way to characterize the halophytes in the metal/metalloid conditions. Characterization helps in understanding the changes occur in the plants [71].

Isozyme

Isozymes are used to know the variation in the plant species and used to measure the intragenic variation through polymorphism at loci [72]. Isozymes along with PCR based DNA markers are used create genetic map of Alfa Alfa plant. It has eight linkage groups that represents the haploidy of the plant *Medigo* species [73]. In plants primarily wild emmer wheat, *Triticum dicoccoides*, and wild barley, *Hordeum spontaneum*, the progenitors of cultivated wheats and barley, respectively isozymes are used to detect quantitative genetic analysis [74]. Isozyme markers with four different enzymes have been used for genotype detection in hill bamboo *Sinarundinaria anceps.* This bamboo is used as variety [48]. Isozymes are used to distinguish the bamboo species at generic, specific and sub-specific level. But there are some limitations also for the isozyme use as it depends on the season and growth conditions so results also vary and it also give very low resolution below species level [28].

Allozyme

Allozymes are single gene markers that are used to measure the genetic diversity in the plant species. Plant allozymes were used to know the variation in the gene of the crop plants and non-crop plants and it was found that crop plants have more genetic diversity than non-crop plants [75]. Allozymes are used in many plant species to know the genetic diversity of the plant as in *wolffia* species [76], genus *Cypripedium* from Russia [77], etc.

RFLP Markers

Phenotypes were the basis for the plant breeding improvement but now breeders use Restriction Fragment Length Polymorphism (RFLP) linkage maps to improve the variety of plants. RFLP maps are also used to clone the gene, the product of which is unknown [78]. RFLP were also used with RAPD marker to evaluate the genetic diversity among crop plant germplasm in the cruciferous family and it was found that RFLP marker is more reliable than RAPD marker for estimating the genetic relationship for more than one species [79]. Identification of bamboo species is difficult due to long flowering period. RFLP markers help in the analysis of germplasm of the bamboo species. Random probes were taken from the library of *Phyllostachys nigra Pst*I and were screened showing huge variation in the RFLP fragments and species – specific patterns were found [53]. RFLP markers are used in the differentiation of the chloroplast DNA in the *Gigantochloa* species of bamboo and two cpDNA lineages were obtained with different fragments of the restriction enzyme [80]. Many advances are there now in the taxonomy of bamboo in which different molecular markers are used but RAPD-RFLP method is rarely used. It is cost effective method and are used to know the genetic variation among the bamboo species in Brazil [41].

PCR Based DNA Markers

RAPD

Advancement in molecular biology technology has led the development of different molecular markers for the detection of genetic polymorphism. Random Amplified Polymorphic DNA is among them. It is based on PCR technique and have low expenses. Prior knowledge of DNA is also not required in the RAPD case [81]. Genetic relationship in muscat grapevines were analysed using RAPD markers and 484 polymorphic bands were obtained that was suitable to determine the coefficient of similarity in grapevine species [82]. RAPD were also used to analyse the genetic diversity among 5 genera of Indonesian bamboo resulting in 86.21% polymorphic bands among the 25 species of 5 genera [43]. Bamboo is widely used in Indonesia and 8 different species were taken from Bellabori Village, Parangloe District, Gowa Regency and Indonesia. 99 different DNA were taken. Polymorphic bands were OPP-08, OPA-15, OPC-11, and OPA-05 primers and the average diversity were 0.47 [83]. Northeast India is also a rich source of bamboo and RAPD markers were used to determine the genetic relationship in 30 species of bamboo among which 79.51% polymorphism were obtained. The 80% similarity were obtained in the species [84].

AFLP

Amplified fragment length polymorphisms are the rapid markers that are based on PCR techniques and are now rapidly used for systematics, DNA fingerprinting and pathotyping. They have high replicability and are easy to use [85]. AFLP markers have been used to evaluate the genetic diversity in *Elymus tangutorum* from Qinghai-Tibet and northwest China. 24 accessions were taken and were evaluated with AFLP marker [86]. Four genera of bamboo (*Bambusa*, *Dendrocalamus*, *Gigantochloa* and *Thyrsostachys*) were analysed by using AFLP markers with 8 primers on 15 species of bamboo and result showed that unique pattern obtained are able to distinguish between different species [87]. 8 AFLP and 42 RAPD were used to analyse the diversity among industrially important reed bamboo (*Ochlandra travancorica Benth*) species in Kerala region of India. Total 50 primers were used that produce 914 polymorphic loci and the polymorphism obtained are useful to know the source of germplasm for industrial use [59]. AFLP markers are used to know the relationship between the North American *Arundinaria gigantean*. Three species were used and their genetic variation and molecular hybridization were analysed [88].

SSR

Simple Sequence Repeat (SSR) markers are a great tool for many purposes but it is less used or used in some important crops due to its high labour work and high cost [65]. It is used to check the transferability to SSR markers to the wheat, rye and triticale and the transferability of the SSR from wheat to rye was 17% whereas from rye to wheat it was 25%. 58% and 39% was the transferability were of triticale to wheat and rye [89]. SSR markers have been used to know the genetic variability of the Indian rice plants to improve the genetic variation of the rice [65]. It is important to know the genetic variation to improve the breeding of the plant. 36HvSSR markers were used to assess the genetic diversity of the Indian variety of the rice that results in the 0.29 PIC value [90] 120 rice markers have been used to evaluate the transferability of the SSR markers to 21 different bamboo species. It was found that transferability were 68.3% from rice to wheat. Markers on the chromosome 7 and chromosome 1 have the highest and lowest transferability respectively concluding that rice SSR markers can be a good source for the markers [91]. Few SSR markers are present in the bamboo species. So to know the genetic diversity of the bamboo species 98 SSR markers from rice and 20 EST derived SSR markers from sugarcane were taken that results in the (44.9%) rice and 15 (75%) sugarcane SSR primer amplification in 23 bamboo species. Complex amplification were seen in the bamboo species and it was concluded that to know the phylogeny and genetic diversity SSR markers from the rice and sugarcane are a good tool [64]. Inexpensive SSR markers were derived from the gene discovery programs that provides an easy tool for marker development. From the *Bambusa oldhamii* database 10 EST-SSR markers were searched and were used to understand the genetic relation between bamboo species [90].

ISSR

PCR based DNA markers are in great demand now a days due to their simplicity and require less amount of sample. Inter Simple Sequence Repeats (ISSRs) markers are one of them. They produce amplification with microsatellite markers and a better tool for genome printing [92]. ISSR markers were used to analyse the genetic diversity in *Psammochloa villosa* (Poaceae) using 84 primers. Out of 84 primers, 12 primers produce highly reproducible bands. 173 DNA fragments were produce with 70.5% polymorphism indicating genetic diversity at species level [93]. ISSR markers were also used to evaluate the genetic diversity in the *Dendrocalamus giganteus* species of bamboo so that they can improve the breeding and can conserve the germplasm. It is a highly valuable species of bamboo found in China's Yunnan and Southeast Asia. Seven primers were used that generated 140 bands with 88.57% polymorphism. Low genetic diversity were observed within the species whereas high level of diversity were observed among population [61]. ISSR markers were also used to know the genetic diversity in the Northeast bamboo *Melocanna baccifera* (Roxb.) Kurz. It is an economically important bamboo in the Northeast India. Seven samples were taken from the population and district Manipur. Analysis shows that genetic diversity of significant level within the population [10]. Gujrat region also contain variety of

bamboo. Among them 6 bamboo species were taken and analyse for their genetic diversity. Seven ISSR primers were used among which 62 band obtained were polymorphic and 25 bands were monomorphic. Result from the analysis not only helps in improving varieties but also helps in classification of the species [94].

CONCLUSION

Bamboo, a golden grass is known for its thousands of uses. It is a perennial grass that mostly grows in warm and moist climate but there are many varieties of the bamboo that can also grow in the Himalayan regions and cold climate. The bamboos are mostly used for non-timber purposes. Many species of bamboo are edible in nature. It is consumed in different forms and have lots of health benefits. There are several medicinal uses of bamboos plant including antibacterial and antifungal. As an economically important species, demands of bamboo are high and its traditional production methods are not appropriate to meet the demands. Therefore, tissue culture technology can be applied to fulfil the requirements. Tissue culture allow growing the plant aseptically. For the conservation of the bamboo, the study of genetic variation plays an important role. Despite of many studies, our knowledge of genetic relationships and genetic diversity in bamboo is still limited because of the inadequate phenotypic variations and irregular flowering. Therefore, by using molecular markers we come to know the diversity between the different varieties. Different molecular markers are being used to study the genetic variation in the sample that will support in the conservation, improvement of the plant variety. Among all, RAPD and ISSR are the most powerful markers for genetic diversity studies in bamboo.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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