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Unlocking Bamboo Genetics: A Review of Molecular Markers and Marker-Assisted Selection

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ABSTRACT: Bamboo has attracted significant attention in research and development due to its rapid growth, environmental benefits, and wide range of industrial applications. Traditional breeding methods, however, have been largely inefficient for bamboo improvement because of its long flowering intervals, large genome, and complex genetic structure. To overcome these challenges, molecular markers and Marker-Assisted Selection (MAS) have emerged as powerful approaches, enabling more precise and efficient enhancement of desirable traits. Molecular markers, which are specific DNA sequences associated with important characteristics, allow researchers to assess genetic variation at a deeper level. Their use in bamboo has strengthened efforts in breeding, genetic analysis, and conservation of germplasm. By incorporating these tools, breeding programs can more accurately identify and select superior traits, thereby improving both the speed and success rate of developing improved varieties. The integration of MAS and molecular marker technologies holds great promise for transforming bamboo cultivation and production. These approaches enhance selection accuracy and reduce the time required to develop high-performing varieties. Although some challenges remain, ongoing advancements in genomics, marker discovery, and high-throughput technologies are expected to further improve their effectiveness and applicability. Combining genetic resources with MAS is accelerating the development of superior bamboo varieties, contributing to increased productivity and sustainability worldwide. This progress is likely to enhance bamboo's ecological and economic importance, particularly in industries such as paper production, bioenergy, and construction. Continued investment in genomic resources, marker development, and advanced genotyping techniques will be crucial for overcoming current limitations and ensuring bamboo remains a valuable and sustainable resource in the future.

Keywords: Bamboo, Molecular marker, Marker-Assisted Selection, Germplasm, Genetic variation.

INTRODUCTION

Bamboo is of paramount importance as a forest resource that promises ecological, economic and social benefits worldwide. Bamboo is a fast-growing woody grass with long juvenility which can be used as an alternate source of wood, textile, fuel and food. Bamboos are rightly called 'green gold of the forest' as they have contributed to the growth of rural livelihood apart from supporting the urban sector. It has also earned the title of 'vegetable steel' as its wood has high mechanical strength, toughness, moderate stiffness and flexibility. It has been used in rural areas for making handicraft products, as construction material in making houses and even as food. In the current scenario it has emerged as one of the most promising renewable resources with multiple uses such as for fuel, pulp, paper, textile, plywood and food. According to an estimate, 20 million tons of bamboo are required per annum for commercial ventures (Scurlock *et al*., 2000). Besides providing ecological solutions to global climate change through its carbon dioxide sequestration ability, about 2.5 billion people worldwide depend on bamboo (Lobovikov *et al*., 2007; Peng *et al*., 2013). Bamboo is one of the few species that can provide a solution to the conglomerated challenges of global climate change and the requirement of alternative renewable sources of energy. Because of the multidisciplinary uses of bamboo, there is an urgent need to develop varieties suitable for specific applications. It is seen as a potential substitute for plastic and thus the Chinese government and International Bamboo and Rattan Organization (INBAR) had launched the "Bamboo as a substitute for plastic initiative". This was done in order to reduce plastic pollution, carbon emission and promote sustainability.

Bamboo breeding faces new challenges in wake of the current environmental crisis and climate change where we need to develop varieties that are suitable for replacing plastics. Owing to its lengthy vegetative growth and uncertain flowering period the breeding methods used in bamboo differ slightly from the ones used in common crops. Bamboo breeding has undergone four parallel stages *i.e.* selective breeding (era 1.0), traditional breeding (era 2.0), molecular breeding (era 3.0) and design breeding (era 4.0), with all these eras overlapping each other.

Selection breeding simply means selecting for individuals with desirable traits in order to develop superior varieties. This has improved certain ornamental traits in bamboo. For example, 21 variants of Moso bamboo (*Phyllostachysedulis*), such as *P. edulis* f. *abbreviata*, *P. edulis* f. *anjiensis*, *Phyllostachysedulis* f. *bicolor* and *P. edulis,* 'Pachyloen', have been selected, and changes in stem color have been observed, including the golden wire, green stripe, yellow stripe, golden stripe, yellow groove and green groove types (Zeng *et al*., 2018). *P. edulis* 'Pachyloen' with its thickened stalk wall, is highly valued for its strength and durability, making it suitable for construction and furniture making (Shen *et al.*, 2020).

Traditional breeding involves employing the primary methods of crop improvement *i.e.* hybridization and mutation breeding. Lengthy vegetative growth in bamboo hampers breeding efforts, along with uncertain flowering period. These obstacles have been overcome by inducing flowering in bamboo through tissue culture system and pollen preservation. Hybridization has been performed between different bamboo genera and species, such as crossing *Bambusa textilis* with *B. pervariabilis* to obtain hybrids with tall culms, strong sprouting abilities and good wood properties, as well as hybrids with long fibers suitable for paper making (Zhang and Chen 1980; 1986). Hybridization of *B. pervariabilis* and *Dendrocalamus daii* produced hybrids suitable for paper making (Wang *et al*., 2005). Hybrids of *B. pervariabilis* and Mabamboo are excellent bamboo varieties for both fresh consumption and processing (Wang *et al.*, 2020).

Molecular breeding is a recent advancement that employs molecular markers and genetic engineering technologies for improving traits in bamboo. Molecular marker-assisted breeding has been widely used in bamboo genetic diversity studies, including taxonomic classification, systematic evolution and germplasm identification (Dev *et al.*, 2020; Ramakrishnan *et al*., 2020; Yeasmin and Nasim 2021). Design breeding is a promising approach for achieving precisely targeted improvement of plant traits by integrating technologies such as gene editing, biotechnology, omics technologies and artificial intelligence (Hartung and Schiemann 2014). There is a dearth of genetic information regarding ornamental and economic traits in Bamboo. Genetic information available through genomic studies is limited to a few species of bamboo, which is not enough to carry out targeted improvement in desirable traits. Thus, continuous effort in the direction of genome studies is required to ensure improvement through breeding in this tree species.

Taxonomy using molecular marker in Bamboo

Taxonomy seeks to systematically classify taxa and create reliable identification keys that reflect their evolutionary history. In the case of bamboo, traditional taxonomy has largely relied on morphological characteristics, but this approach has encountered significant challenges. These include superficial classifications, incomplete herbarium specimens, and a heavy reliance on reproductive traits that are often difficult to assess due to the irregular flowering cycles of bamboo. To overcome these issues, molecular markers have been increasingly adopted in bamboo taxonomy, providing valuable insights into genetic diversity, phylogenetic relationships, and species identification. This review examines the latest developments in the use of molecular markers for bamboo taxonomy, emphasizing their applications and contributions to the unlocking bamboo genetics.

Molecular Markers in Bamboo Taxonomy

Most of the existing classifications for bamboo have largely relied on various morphological traits, but there is an urgent need to evaluate the realism of these systems. Stapleton (1997) identified several key limitations of traditional morphology-based classifications: (1) These classifications are often superficial, as similarities are frequently emphasized over differences. (2) Reproductive characteristics have been prioritized under the assumption that they carry greater evolutionary significance than vegetative traits. However, the importance of many vegetative features, such as rhizome or branch patterns, was recognized later, leading to incomplete early herbarium specimens. (3) In many instances, artificiality has been exacerbated by the tendency to consider characteristics in isolation rather than in groups.

Vegetative characteristics are essential for identifying woody bamboos because their irregular flowering makes reproductive features rarely available. Herbarium samples often lack enough detail, so identification mainly depends on vegetative traits, which still need improvement. The classification of woody bamboos at genus and species levels remains unclear, with many species identified only by vegetative features, new ones still being discovered, and several yet to be formally described, especially in South and Central America (Clark *et al*., 2007; Triplett *et al*., 2006).

Molecular data have become increasingly valuable in resolving different aspects of plant taxonomy. Significant advancements have been made in bamboo research. A key difficulty with any molecular approach is identifying the correct taxonomic level where it provides the most meaningful insights and ensuring it aligns with morphologically defined taxonomic categories. Improved knowledge of bamboo genetics has resulted from the creation and application of numerous molecular markers. In bamboo research, some often used markers are:

1. Restriction Fragment Length Polymorphism (RFLP)

RFLP markers involve detecting differences in restriction enzyme recognition sites. Friar and Kochert (1991, 1994) utilized RFLP to assess the phylogeny of 61 accessions and 20 species of Phyllostachys, supporting the presence of two distinct sections but disagreeing on the placement of P. nigra (Friar & Kochert 1994). Despite its early promise, the regular use of RFLP in bamboo has been limited due to the requirement for large DNA amounts and radioactive isotopes.

2. Randomly Amplified Polymorphic DNA (RAPD)

RAPD markers use a single arbitrary primer to amplify random DNA segments. Gielis *et al*. (1997) used RAPD to study the phylogenetic relationships among 73 genotypes of Phyllostachys, revealing that P. nigra belongs to the section Phyllostachys. However, the reliability and reproducibility of RAPD remain questionable. Developed in 1990 by Williams *et al*., RAPD has been applied to bamboo studies since 1997**.** This technique is known for its cost-effectiveness, rapid processing, and independence from prior knowledge of the plant genome, which contributes to its popularity. RAPD markers are useful for determining genetic relationships among varieties and species.

Several studies have leveraged RAPD markers to explore genetic diversity within bamboo. Nayak *et al.* (2003) utilized ten RAPD primers to analyze twelve bamboo species, including *Bambusa vulgaris* and *Dendrocalamus giganteus*. They identified 137 distinct polymorphic DNA fragments across the species studied. Shalini *et al*. (2013) reported 21 RAPD markers, out of them seven exhibited significant polymorphisms useful for differentiating ten bamboo genotypes, such as *Bambusa tulda* and *Dendrocalamus asper*. Desai *et al.* (2015) used RAPD markers to differentiate thirteen Indian bamboo genotypes, revealing over 60% polymorphism. Tiwari *et al.* (2019) demonstrated the utility of RAPD in assessing genetic diversity among ten genotypes of *Thamnocalamus spathiflorus*, showing its effectiveness in estimating both close and distant genetic relationships. In Indonesia, Annisa *et al.* (2019) evaluated 25 species from five bamboo genera with 40 RAPD markers and they have found that 24 primers produced 86.21% polymorphic bands. Eevera *et al.* (2008) used ten RAPD markers to analyze Bambusa and Dendrocalamus genotypes, showing that *D. brandisii* clustered with Bambusa, while *D. giganteus* was more distantly related. These studies affirm RAPD markers' utility in distinguishing genetic variation among bamboo taxa.

Moreover, RAPD's derivative technique, RAPD-RFLP, enhances the method by combining random amplification with restriction enzyme digestion and gel electrophoresis. This approach was used to analyze genotypes of thirteen bamboo taxa, including Bambusa vulgaris and Phyllostachys edulis, with the RAPD products digested by three enzyme combinations (MspI, HindIII/HaeIII, and HinfI/RsaI). This method resulted in a 79% increase in polymorphic bands compared to the use of twelve RAPD primers (Konzen *et al*., 2017). In summary, while RAPD markers offer a practical and effective means of studying bamboo genetic diversity, their efficiency can vary. The integration of RAPD with techniques like RAPD-RFLP enhances the resolution of genetic analyses, contributing to a more comprehensive understanding of bamboo taxonomy.

3. Sequence Characterized Amplified Region (SCAR)

SCAR markers, derived from RAPD, offer better reproducibility with higher annealing temperatures. SCARs are co-dominant and useful for genotype identification, particularly at the seedling stage. Das *et al*. (2005) developed SCAR markers for *B. balcooa* and *B. tulda* to aid the paper and pulp industry in accurate species diagnosis.

4. Amplified Fragment Length Polymorphism (AFLP)

AFLP markers combine RFLP and PCR techniques, generating dominant markers. Loh *et al.* (2000) demonstrated the efficiency of AFLP in measuring genetic relationships among 15 bamboo species, revealing the polyphyletic nature of the genus Bambusa. AFLP has also been used to assess genetic diversity in woody American bamboos, such as Guadua angustifolia (Marulanda *et al*., 2002).

5. Simple Sequence Repeats (SSR)

SSR or Microsatellite markers, are highly effective tools for analyzing genetic diversity across various plant species. These markers consist of short tandem repeats, typically 1-6 nucleotides in length, and are designed using primers from conserved genomic regions adjacent to the repeat sequences (Das *et al*., 2008). SSR markers are extensively utilized in genetic diversity studies, classification, and genetic mapping for detecting crucial plant traits. Since 1997, SSR markers have been successfully applied to the study of Phyllostachys bamboo species (Lai and Hsiao, 1997). Nayak and Rout (2005) studied the 18 bamboo species from the genera Bambusa, Dendrocalamus, Dinochela, Cephalostachyum, Sasa, Shibata, and Arundinaria were examined using six SSR markers developed from *Bambusa arundinacea*. Three of these markers were polymorphic, and the researchers concluded that these polymorphic loci could be valuable for genetic studies. Meena *et al.* (2019) utilized 17 SSR primer pairs, derived from *Dendrocalamus latiflorus* and *Bambusa arundinacea*, to assess the genetic diversity of 19 natural populations of *Dendrocalamus hamiltonii*, a commercially significant bamboo species found in the Northeast Himalayas. Additionally, Sharma *et al*. (2008) analyzed the genetic diversity of 43 bamboo accessions, representing 23 species from the Dendrocalamus, Bambusa, Phyllostachys, Ochlandra, Sasa, and Melocanna genera, using 42 SSR markers. Their study revealed 73% variation among species, though diversity within individual species was relatively low. Furthermore**,** Cai *et al*. (2019) used 54 EST-SSR (expressed sequence tag-simple sequence repeats) markers to investigate the genetic profiles of 16 Lei bamboo (*Phyllostachys violascens*) varieties and nine additional Phyllostachys species, including *P. glabrata, P. verrucosa, P. bambusoides, P. aurea, P. edulis, P. virella, P. rivalis, P. parvifolia, and P. nidularia*. These markers were well-suited for analyzing genetic segregation within P. violascens varieties and among different Phyllostachys species.

6. Inter Simple Sequence Repeat (ISSR)

ISSR markers have become a popular tool for investigating genetic diversity in various plant species, such as *Sorghum bicolor*, banana (Godwin *et al*., 1997), Lilium (Žukauskienė *et al*., 2014), Jasminum (Akhtar *et al*., 2021), Ziziphus spina-christi (Alansi *et al*., 2016), *Cycas guizhouensis* (Xiao *et al*., 2004), and *Balanites aegyptiaca* (Abdelaziz *et al*., 2020). Their effectiveness has also been demonstrated in bamboo species classification. For instance, in North-East India, genetic diversity was assessed among 93 individuals of the economically significant *Melocanna baccifera* across seven locations using five different ISSR markers. The findings revealed substantial genetic variation within the populations, with 88.37% polymorphic bands observed (Nilkanta *et al*., 2017). In another study, twelve ISSR primers and four EST-based random primers were utilized across 22 accessions from nine Bambusa species, five Dendrocalamus species, two Pleioblastus species, and one species each of Melocanna, Oxytenanthera, Phyllostachys, Thyrsostachys, Schizostachyum, and Sasa, resulting in the amplification of 220 polymorphic bands (Mukherjee *et al*., 2010). Additionally, Amom *et al*. (2018) analyzed the phylogenetic relationships among 15 different bamboo species from North-East India, including *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*, using 10 ISSR markers. Their study identified genetic variation among these species, categorizing them into two distinct cluster groups. Similarly, Yang *et al.* (2012) conducted an ISSR marker analysis on 12 accessions of *Dendrocalamus membranaceus* from Yunnan Province, finding that 98.71% of the loci were polymorphic.

Challenges of using Molecular Markers in Bamboo Taxonomy

Although molecular markers have greatly improved bamboo taxonomy, challenges persist due to complex genomes, polyploidy, and limited genomic data. Expanding resources like reference genomes and transcriptomes, along with advances in next-generation sequencing, will enable more precise genetic analysis. Combining molecular, ecological, and morphological data will offer a more complete understanding of bamboo diversity and evolution. Overall, tools such as RFLP, RAPD, AFLP, SSR, and others have transformed bamboo research, and continued technological progress will further strengthen classification, conservation, and sustainable use of these vital plants.

Role of Marker Assisted Selection in Bamboo Breeding

MAS has opened new avenues for breeders which has helped in overcoming many shortcomings of conventional breeding. As compared to conventional breeding, molecular breeding allows setting of goals, shortening breeding cycles, accelerated breeding processes, consumes less time and is especially important for long duration bamboo. Although, only a few important genes from the breeding point of view have been identified but marker based breeding methods would change the face of bamboo breeding in the near future.

Many markers such as SSR and single nucleotide polymorphism (SNP) have aided in the evaluation of genetic and phylogenetic variation in bamboo species. A whole genome resequencing of 427 Moso bamboo individuals has been carried out by Zhao *et al.* (2021) which identified 104 candidate genes associated with cell wall, carbohydrate metabolism and environmental adaptation through genome wide association analysis of nine important traits. The first genome draft of bamboo has laid down the foundation for genetic engineering approaches for enhancing desirable traits in bamboo. For example, Agrobacterium EHA105 carrying the bacterial CodA gene under the control of the Rd 29A inducible promoter was used by Zhang *et al.* (2012); Qiao *et al.* (2014) to generate transgenic plants with improved cold tolerance. Recently, the maize leaf color gene (Lc) has been successfully transformed into Ma bamboo which led to the development of transgenic bamboo plants with anthocyanin accumulation.

To develop bamboo with both ornamental and economic values, there is a need to modify culm morphology along with culm and leaf color. The overexpression of certain genes such as RUBY which is involved in betalain synthesis and bHLH transcription activator gene Lc, can make this possible. RUBY gene has been successfully expressed in leaf sheaths of Moso bamboo, where vaccum infiltration and injection were used to improve the efficacy of Agrobacterium mediated infection. The glucuronidase gene (GUS) and hygromycin resistance genes have been transformed into calli with 68.5 *per cent* expression rate. Gene gun bombardment method was used by Hu *et al.* (2017) for transferring the GUS gene into mature embryos of Moso bamboo and detected its expression in bamboo seedlings. Zhang *et al.* (2021) developed a subcellular localization system suitable for Moso bamboo by using PEG mediated gene transformation to introduce a set of multicolored fluorescent organelle marker genes into protoplast cells from stems and leaf sheaths.

In the process of engineering fiber plywood from bamboo, internodes with a unidirectional fiber structure are desirable as opposed to short nodes that have weaker mechanical properties. This makes long internodes with high fiber content ideal materials for paper and textile production. This can be done by manipulation of enzymatic activity in metabolic pathways, transcription factors involved in internode elongation. Overexpression of GA responsive gene DlmGRG1 (Ye *et al.,* 2020) and flavin monooxygenase gene YUCCA (Bai *et al.* 2022) can make this possible.

With the rising demand for bamboo as a sustainable and renewable resource, breeding objectives in bamboo are centered on enhancing yield, wood properties and adaptation to diverse geographical conditions. In the coming future bamboo breeding will focus more keenly on fiber length, internode length, wall thickness, regulation of lignin and cellulose content for paper making and replacing plastics.

Genetic diversity

Genetic diversity in bamboo has been extensively studied using molecular markers, which are reliable tools for detecting DNA-level variation. Due to bamboo's long flowering cycles and limited morphological differences, techniques such as RAPD, AFLP, SSR, and ISSR are widely used to assess genetic relationships, population structure, and variability among species. These markers help in identifying superior genotypes, conserving germplasm, and supporting effective breeding programs (Das *et al*., 2005; Nayak *et al*., 2003; Sharma & Negi (2010). Studies have shown that molecular markers provide accurate insights into genetic diversity and phylogenetic relationships in bamboo, making them essential for sustainable management and improvement of bamboo resources (Lalhruaitluanga *et al*., 2014).

The Importance of genetic diversity in bamboo

Bamboo represents a distinct and varied group of woody grasses with substantial ecological and economic value, especially in areas like Asia, Africa and the Americas. The genetic diversity within bamboo species is crucial for several reasons:

1. Adaptation to environmental changes: Bamboo species occupy a broad spectrum of climatic environments, ranging from tropical rainforests to temperate zones. The genetic diversity within these species equips them with the ability to adapt to a variety of environmental conditions, including fluctuations in temperature, moisture and soil types. This adaptability is essential for bamboo's survival, particularly as climate change is projected to increase the frequency and intensity of extreme weather events, which could alter the habitats where bamboo currently thrives (Nijjer *et al*., 2018).

2. Disease and pest resistance: The genetic diversity within bamboo populations offers a broader array of genetic resources for defending against diseases and pests. Individual plants within a population may exhibit different levels of resistance to pathogens or herbivores, which helps mitigate the risk of a single outbreak severely impacting the entire population. This genetic variability is especially crucial in bamboo cultivation, which often relies on monocultures and is therefore more susceptible to widespread infestations or infections (Zhang *et al*., 2013).

3. Population health and stability: Populations with high genetic diversity are typically more resilient and capable of sustaining their health and reproductive success across generations. This genetic variability is crucial for enabling bamboo populations to recover from disturbances like habitat loss or overharvesting, which frequently occur in areas where bamboo is intensively exploited for economic purposes (Bystriakova *et al*., 2004).

4. Economic value and utilization: Bamboo serves as a vital resource globally, with applications spanning construction, paper production, textiles and food. The genetic diversity within bamboo species facilitates the development and selection of varieties with beneficial traits, including accelerated growth rates, enhanced stem strength and flexibility and improved resistance to environmental stresses. This genetic variability not only aids in the sustainable management and cultivation of bamboo but also fosters innovation across industries that depend on this adaptable plant (Liese and Köhl 2015).

Threats to genetic diversity in bamboo

Despite its importance, the genetic diversity of bamboo is under threat from various factors:

1. Habitat loss and fragmentation: Activities such as deforestation, agricultural expansion and urbanization have resulted in the destruction and fragmentation of bamboo habitats. This fragmentation disrupts gene flow between populations, creating smaller, isolated groups that are more vulnerable to inbreeding and genetic drift. Over time, these factors can lead to a decrease in genetic diversity within bamboo populations (Bystriakova *et al*., 2004).

2. Overharvesting: The growing economic demand for bamboo products has resulted in the overexploitation of certain bamboo species, especially those with valuable traits. This excessive harvesting can lead to reduced population sizes and the loss of rare alleles from the gene pool, which further decreases genetic diversity within these species (Liese and Köhl 2015).

3. Climate change: With rising global temperatures and shifting weather patterns, bamboo populations may need to adjust to new environmental conditions. If a population has insufficient genetic diversity, its capacity to adapt may be limited, potentially resulting in declines or even local extinctions (Nijjer *et al*., 2018).

Conservation and management of bamboo genetic diversity

To protect the genetic diversity of bamboo, it is essential to implement conservation strategies that include both *in situ* (within natural habitats) and *ex situ* (such as in botanical gardens or gene banks) approaches. These efforts should focus on sustaining large, genetically diverse populations, safeguarding critical habitats from degradation, and encouraging the sustainable management of bamboo resources. Additionally, studying molecular genetic diversity offers critical insights into the genetic composition and variability of bamboo species. Molecular techniques and tools are valuable for evaluating genetic variation and understanding the genetic relationships among bamboo populations. These molecular tools can help identify genetic bottlenecks, monitor gene flow and evaluate how environmental changes affect genetic diversity. Continued research using these techniques is essential for developing effective conservation strategies and enhancing the genetic resilience of bamboo species (Bystriakova *et al*., 2004; Zhang *et al*., 2013).

Applications of genetic markers in bamboo

Genetic markers play a crucial role in evaluating the genetic diversity within and among bamboo species. By examining variations in specific DNA sequences, researchers can gauge the extent of genetic diversity across different bamboo populations. This genetic insight is vital for identifying populations with unique genetic characteristics that may be significant for conservation efforts and breeding programs. Different types of molecular markers provide valuable tools for these assessments. For instance, AFLP and SSR markers have been extensively utilized in bamboo research. AFLP markers, known for their ability to generate a large number of polymorphic bands, have been particularly useful for analyzing genetic variation and differentiating between bamboo populations. Gielis *et al*. (2001) employed AFLP markers to investigate the genetic diversity of Phyllostachys species, uncovering substantial genetic differentiation among populations. Their study highlighted how AFLP markers can reveal intricate patterns of genetic variation within bamboo species. Similarly, SSR markers, which are also known as microsatellites, are valuable for assessing genetic diversity due to their high level of polymorphism and ability to provide detailed information on allele frequencies. Zhang *et al*. (2013) used SSR markers to evaluate the genetic diversity of Bambusa species, finding significant polymorphism across various populations. Their research demonstrated the utility of SSR markers in capturing the genetic variability and population structure of bamboo species. In addition to AFLP and SSR markers, other molecular techniques such as SNPs and RAPD have also been employed to study bamboo genetic diversity. SNPs provide a high resolution of genetic variation, while RAPD markers offer a rapid and cost-effective approach for detecting genetic differences.

Understanding population structure

Genetic markers play a key role in revealing how bamboo populations are structured and how genetic variation is distributed within and between them. They help track gene flow, migration, and past population changes—insights that are essential for conservation and sustainable management. For instance, RAPD markers have been used in *Dendrocalamus* species to uncover genetic relationships, diversity patterns, and connectivity among populations. Such analyses highlight gene exchange, detect barriers to movement, and reflect historical influences, ultimately guiding effective strategies to preserve and manage bamboo genetic resources.

Phylogenetic studies and species identification

Genetic markers are essential tools in phylogenetic studies, which aim to reconstruct the evolutionary relationships among species. In bamboo research, markers such as chloroplast DNA and SSR have been instrumental in elucidating the taxonomic classification of various bamboo species and genera. For instance, Hodkinson *et al*. (2000) utilized chloroplast DNA markers in their phylogenetic study to explore the evolutionary relationships among temperate bamboo species. Their research identified distinct lineages within the genus, enhancing our understanding of bamboo evolution and classification. By analyzing chloroplast DNA, they were able to clarify the evolutionary history and resolve taxonomic ambiguities among bamboo species. In addition to phylogenetic studies, genetic markers are critical for species identification and authentication, particularly in cases where morphological traits alone are insufficient to differentiate closely related species. SNPs are particularly valuable for this purpose due to their high resolution and specificity. SNP markers enable precise identification of bamboo species and the detection of hybrids, which is crucial for both conservation and commercial purposes.

Marker-Assisted Selection in breeding program

MAS is a powerful technique that leverages genetic markers to identify and select individuals with desirable traits for breeding programs. By using MAS, breeders can detect superior genotypes at an early stage in the breeding cycle, which accelerates the selection process and enhances the accuracy of trait selection. In bamboo breeding, Marker-Assisted Selection holds noteworthy potential for improving various traits such as growth rate, disease resistance and wood quality. The SSR markers have been employed to identify genetic loci associated with these important traits in *Bambusa* species. The identification of these markers facilitates the development of new bamboo cultivars with enhanced characteristics. By using SSR markers linked to specific traits, breeders can more efficiently select individuals with the desired attributes, leading to improved breeding outcomes and more effective cultivation practices. The application of MAS in bamboo can lead to more rapid advancements in breeding programs, enabling the development of bamboo varieties that are better suited to changing environmental conditions, resist diseases and meet commercial demands for superior wood quality. This approach not only streamlines the breeding process but also contributes to the sustainable management and utilization of bamboo resources.

MAS eliminates the need for labor-intensive and time-consuming phenotypic assessments by using molecular markers associated with significant features to identify individuals with favorable genetic profiles. Refining bamboo genetic improvement using MAS are:

1. Multifactorial features: When breeding bamboo, it's common to select several features at once, including growth rate, pest resistance, and wood quality. Breeders can concentrate on the main genes causing these features while taking environmental influences into account thanks to MAS. More accurate selection is achieved when molecular markers are used in conjunction with conventional phenotypic selection methods such as assessing resistance and growth in field trials (Zheng *et al*, 2024).
2. Genome-Wide Association Studies (GWAS): As phenotypic and genotypic data have accumulated, GWAS has developed into a potent tool for locating loci associated with intricate bamboo features. By identifying the genes causing phenotypic variances, this technique improves MAS's capacity to forecast and choose desirable features (Le *et al*, 2023).
3. Targeted qualities: CRISPR and MAS can be used together to modify or produce new genetic variants for qualities like abiotic stress tolerance or insect resistance that are hard to select for using conventional breeding methods or MAS alone (Huang *et al*, 2022).
4. Large-scale genotyping of bamboo populations is now possible thanks to the growing affordability and accessibility of the genotyping by sequencing (GBS) technique. Important properties including wood quality, pest resistance, and drought resistance can be linked to genome-wide markers that GBS can detect (Yeasmin *et al*, 2015).
5. Reference Genomes: Several bamboo species have high-quality reference genomes. By providing comprehensive knowledge about the genes governing characteristics like growth rate, disease resistance, and wood quality, this strengthens the basis for MAS (Zheng *et al*, 2024).
6. Transcriptome Data: The availability of identified genes that are active at different stages of bamboo growth and in diverse environmental conditions and these data help in associating genes with desired characteristics for selection (Wang *et al*, 2020).

Recent Developments in MAS:

1. Disease & Pest Resistance: Molecular markers associated with bamboo resistance features (mites, termites, and fungal infections) have been discovered in recent research. By using these markers in MAS, resistant bamboo plants can be chosen, enhancing sustainable bamboo farming and lowering the need for chemical pesticides (Dey *et al*, 2023).
2. Biomass Yield and Growth Rate: By choosing bamboo plants with exceptional growth traits, MAS can contribute to increased output. For bamboo used to produce bioenergy and building materials, research has concentrated on finding markers associated with high biomass yield and quick growth (Yeasmin *et al*, 2015).
3. Flowering Behavior: One of the biggest breeding challenges for bamboo is its unusual flowering cycle, which happens just once every few decades in many species. In bamboo breeding projects, MAS can be used to choose non-flowering or early flowering cultivars to promote hybridization and seed production (Dutta *et al*, 2023).
4. Enhancing Growth and Biomass Production: MAS speeds up the selection of superior bamboo cultivars by locating markers associated with genotypes that grow quickly or produce large amounts of biomass (Huang *et al*, 2022).
5. Drought and Stress Tolerance: Breeders can choose bamboo plants that can flourish in water-scarce conditions by using markers associated with stress tolerance features. This is a crucial factor in climate change adaptation (Dey *et al*, 2023).
6. Wood Size and Quality: Bamboo plants with superior physical traits, like larger culms or better wood density, are sought after for construction and paper making. MAS assists in identifying these plants (Le *et al*, 2023).

Challenges and future perspectives

Even with the notable advancements, there are still several obstacles to overcome when using MAS and molecular markers in bamboo:

1. Absence of Reference Genome: Although some bamboo species have undergone sequencing, the full potential of MAS is restricted by the lack of a complete reference genome for many bamboo species. However, this gap is anticipated to be filled by increasing genetic research.
2. Development of Species-Specific Markers: Because bamboo species differ greatly in their genetic characteristics, it is challenging to identify markers that are generally applicable. Research is still being done on creating markers for important features or species-specific markers.
3. Cost of Genomic Tools: One obstacle to the widespread use of MAS in bamboo breeding programs is the high expense of high-throughput genotyping, such as SNP genotyping.

4. Phenotyping and Breeding Cycle: Because bamboo has a lengthy reproductive cycle (flowering may happen only once per few decades), MAS is difficult to include into conventional breeding methods. This problem might be lessened by attempts to address it through tissue culture advancements and rapid breeding techniques.

CONCLUSION

Genetic diversity is essential for the adaptability, resilience, and long-term survival of species, as it provides the foundation for evolution. In bamboo, this diversity plays a crucial role in enabling adaptation to varied environmental conditions, resistance to pests and diseases, and maintenance of healthy and stable populations. Considering bamboo's significant ecological and economic value, preserving its genetic variability is vital for its sustainable utilization. However, bamboo genetic diversity is increasingly threatened by habitat loss, fragmentation, overexploitation, and climate change. These challenges necessitate a comprehensive approach that integrates in situ and ex situ conservation strategies, along with sustainable management practices and continued scientific research. Molecular markers have emerged as powerful tools for assessing and managing genetic diversity in bamboo. Techniques such as AFLP, SSR, RAPD, and SNPs allow precise evaluation of genetic variation, population structure, and evolutionary relationships. These methods also support breeding programs through Marker-Assisted Selection (MAS), enabling the development of improved and resilient bamboo varieties. By combining molecular tools with effective conservation strategies, it is possible to protect bamboo genetic resources and ensure their sustainable use. Continued research and strategic efforts are essential to preserve bamboo diversity and maintain its ecological and industrial importance worldwide.

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