

## **Feature Review**

**Open Access** 

# Marker-Assisted Selection in *Camellia* Breeding Programs

Lian Chen, Chuchu Liu ⋈ Institute of Life Sciences, Jiyang Colloge of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China Corresponding email: chuchu.liu@jicat.org Molecular Plant Breeding, 2024, Vol.15, No.4 doi: 10.5376/mpb.2024.15.0019 Received: 30 Jun., 2024 Accepted: 31 Jul., 2024 Published: 15 Aug., 2024 Copyright © 2024 Lu and Wang, This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Preferred citation for this article:

Chen L., and Liu C.C., 2024, Marker-assisted selection in *Camellia* breeding programs, Molecular Plant Breeding, 15(4): 187-197 (doi: 10.5376/mpb.2024.15.0019)

**Abstract** Marker-assisted selection (MAS) has revolutionized the breeding of *Camellia* species, offering enhanced precision and efficiency in developing cultivars with desirable traits. This study provides a comprehensive overview of the advancements and applications of MAS in *Camellia* breeding programs. Key findings highlight the diversity and breeding objectives of *Camellia* species, the types and development of genetic markers, and the implementation of MAS strategies such as Marker-Assisted Backcrossing (MABC), Marker-Assisted Recurrent Selection (MARS), and Genomic Selection (GS). Advances in genomic technologies, including next-generation sequencing (NGS), genotyping-by-sequencing (GBS), genome-wide association studies (GWAS), and CRISPR/Cas9 gene editing, have significantly impacted MAS, enabling more accurate and efficient breeding. Unique insights from case studies demonstrate the practical applications and successes of MAS in enhancing disease resistance in *Camellia japonica*, improving cold tolerance in *Camellia oleifera*, and advancing tea quality in *Camellia sinensis*. This study expects to drive the development of superior *Camellia* varieties, ensuring sustainability and meeting market demands for both ornamental and economic purposes.

Keywords Camellia; Marker-Assisted Selection (MAS); Genomic technologies; Genetic markers; Plant breeding

#### **1** Introduction

*Camellia*, a genus encompassing over 250 species, are economically significant, particularly *Camellia sinensis*, which is the source of tea, one of the most consumed beverages globally (Bali et al., 2013; Tan et al., 2013). Additionally, species like *Camellia oleifera* are valued for their oil production, which has nutritional and pharmaceutical benefits (Tian et al., 2022; Yan et al., 2022). The economic importance of *Camellia* species is underscored by their contributions to agriculture, horticulture, and the global economy.

Traditional breeding methods in *Camellia* species face several challenges due to the plants' perennial nature, long generation times, and high heterozygosity (Bali et al., 2013; Karunarathna et al., 2020; Liu, 2024). These factors make conventional breeding time-consuming and labor-intensive, often requiring many years to develop new cultivars with desirable traits (Bali et al., 2013; Malebe et al., 2021; Jiang et al., 2024). Additionally, the genetic complexity and self-incompatibility of *Camellia* species further complicate breeding efforts (Bali et al., 2013; Yao et al., 2024).

Marker-Assisted Selection (MAS) offers a promising alternative to traditional breeding methods by utilizing molecular markers to select for desirable traits at the seedling stage, thereby accelerating the breeding process (Dubey et al., 2020; Karunarathna et al., 2020). MAS involves the identification and use of specific DNA markers linked to traits of interest, such as disease resistance, yield, and quality attributes (Karunarathna et al., 2020; Malebe et al., 2021). In *Camellia* breeding, various types of molecular markers, including Simple Sequence Repeats (SSRs) and Sequence-Related Amplified Polymorphism (SRAP) markers, have been developed and utilized for genetic analysis and breeding programs (Sharma et al., 2009; Feng et al., 2020; Tian et al., 2022).

This study is to summarize the advancements in MAS techniques and their applications in *Camellia* breeding, assess the effectiveness of MAS in improving yield, fruit attributes, and other important traits in *Camellia* species, identify the challenges and limitations associated with the implementation of MAS in *Camellia* breeding. By synthesizing the existing literature, this study expects to provide a comprehensive understanding of the role of



MAS in *Camellia* breeding, enhance the efficiency and success of MAS in *Camellia*, and explore its potential to revolutionize the development of superior *Camellia* varieties.

## 2 Overview of *Camellia* Species and Breeding Objectives

## 2.1 Diversity and distribution of Camellia species

The genus *Camellia* comprises over 250 species, predominantly distributed in East Asia, particularly in China, Japan, and Korea. These species exhibit significant diversity in morphology, habitat, and ecological adaptability. For instance, *Camellia sinensis* is widely cultivated for tea production (Guo et al., 2024; Zhang et al., 2024), while *Camellia oleifera* is valued for its oil-rich seeds (Sharma et al., 2009; Feng et al., 2020; Tian et al., 2022). The genetic diversity within and among *Camellia* species is substantial, as evidenced by various molecular marker studies, which have revealed high levels of polymorphism and heterozygosity (Sharma et al., 2009; Tan et al., 2013; Tian et al., 2022). This diversity is crucial for breeding programs aimed at improving both ornamental and economic traits.

### 2.2 Breeding objectives for ornamental and economic traits

### 2.2.1 Flower color and shape

Flower color and shape are critical ornamental traits in *Camellia* breeding. The diversity in petal color and flower morphology among *Camellia* species and cultivars is significant, with colors ranging from white to deep red and various shapes from single to double petals. Molecular markers, such as RAPD and SSR, have been used to correlate genetic diversity with these morphological traits, aiding in the selection of desirable phenotypes (Wang et al., 2011).

#### 2.2.2 Disease resistance

Disease resistance is a vital breeding objective, particularly for fungal diseases like blister blight, which can cause substantial crop losses. Marker-assisted selection (MAS) has been employed to identify and incorporate resistance genes into breeding lines. For example, an EST-SSR marker associated with blister blight resistance has been identified in *Camellia sinensis*, facilitating the development of resistant cultivars. The use of molecular markers accelerates the breeding process and enhances the accuracy of selecting disease-resistant plants (Foolad and Panthee, 2012; Miedaner and Korzun, 2012; Karunarathna et al., 2020).

#### 2.2.3 Cold tolerance

Cold tolerance is another important trait, especially for *Camellia* species grown in temperate regions. Breeding for cold tolerance involves selecting genotypes that can withstand low temperatures without significant damage. Genetic studies have shown that cold tolerance is a heritable trait, and molecular markers can be used to identify and select cold-tolerant genotypes in breeding programs (Feng et al., 2020; Tian et al., 2022).

#### 2.2.4 Oil content and quality

For species like *Camellia oleifera*, oil content and quality are primary economic traits. Breeding programs aim to enhance oil yield and improve the fatty acid composition of the oil. Studies have shown that traits related to oil content, such as fruit diameter, seed weight, and oil quality, exhibit high heritability, making them suitable targets for selection. The use of molecular markers, such as SRAP and SSR, has been instrumental in identifying genetic loci associated with these traits, thereby guiding the selection of superior genotypes (Feng et al., 2020; Tian et al., 2022).

## 3 Genetic Markers in Camellia

## 3.1 Types of genetic markers used in plant breeding

## 3.1.1 Morphological markers

Morphological markers are phenotypic traits that can be visually observed and measured, such as flower color, leaf shape, and plant height. These markers have been traditionally used in plant breeding due to their simplicity and ease of observation. However, they are often influenced by environmental factors and may not always accurately reflect the genetic makeup of the plant.



## 3.1.2 Biochemical markers

Biochemical markers involve the analysis of proteins and other metabolites. Isozymes, which are different forms of an enzyme that catalyze the same reaction, are commonly used biochemical markers. These markers can provide more precise genetic information compared to morphological markers but are still limited by environmental influences and the complexity of protein analysis.

### 3.1.3 Molecular markers

Molecular markers are DNA sequences that can be used to identify genetic differences between individuals. They are highly reliable and not influenced by environmental conditions. Several types of molecular markers are used in plant breeding. Restriction fragment length polymorphisms (RFLP): this technique involves the digestion of DNA with restriction enzymes and the separation of resulting fragments by gel electrophoresis. RFLPs are highly polymorphic and co-dominant, making them useful for genetic mapping and diversity studies (Ma et al., 2010). Amplified fragment length polymorphisms (AFLP): AFLP combines the principles of RFLP and PCR to amplify DNA fragments. It is a highly sensitive method that can detect a large number of polymorphisms, making it suitable for constructing genetic linkage maps (Chang et al., 2017). Simple sequence repeats (SSR): also known as microsatellites, SSRs are short, repetitive DNA sequences that are highly polymorphic. They are widely used in genetic diversity studies, linkage mapping, and marker-assisted selection due to their high reproducibility and co-dominant inheritance (Sharma et al., 2009; Li et al., 2021; Tian et al., 2022). Single nucleotide polymorphisms (SNP): SNPs are single base pair variations in the DNA sequence. They are the most abundant type of genetic variation and can be used for high-resolution mapping and association studies. SNP markers are increasingly used in plant breeding due to advances in next-generation sequencing technologies (Yang et al., 2012).

### 3.2 Development of genetic markers for Camellia

### 3.2.1 Marker discovery and validation

The discovery of genetic markers in *Camellia* involves the identification of polymorphic DNA sequences that can be used to differentiate between genotypes. Techniques such as RNA sequencing (RNA-seq) and expressed sequence tag (EST) analysis have been employed to identify SSR markers in various *Camellia* species (Liu et al., 2018; Li et al., 2021; Tian et al., 2022). For instance, in *Camellia japonica*, a total of 28 854 potential SSRs were identified, and 172 primer pairs were synthesized, with 111 found to be polymorphic (Li et al., 2021). Similarly, in *Camellia chekiangoleosa*, 97 510 SSR loci were identified, and the development efficiency of polymorphic SSR primers was 26.72% (Tian et al., 2022).

Validation of these markers involves testing their polymorphism and transferability across different *Camellia* species and accessions. For example, 96 SSR markers were developed for *Camellia sinensis*, and their polymorphism was assessed in 47 tea cultivars, demonstrating high levels of genetic diversity (Liu et al., 2018). Additionally, 74 novel polymorphic EST-SSR markers were developed for *Camellia sinensis*, with observed heterozygosity ranging from 0.000 to 1.000 (Ma et al., 2010).

#### 3.2.2 Linkage mapping and QTL analysis

Linkage mapping involves the construction of genetic maps that show the relative positions of genetic markers on chromosomes. These maps are essential for identifying quantitative trait loci (QTLs) associated with important agronomic traits. In *Camellia sinensis*, a genetic linkage map was constructed using RAPD, AFLP, and SSR markers, covering 1441.6 cM with an average distance of 4.7 cM between markers (Chang et al., 2017). This map provides a foundation for QTL analysis and marker-assisted selection in tea breeding programs.

QTL analysis involves the identification of genomic regions associated with specific traits. For example, in a study on *Lupinus angustifolius*, next-generation sequencing was used to identify SNP markers linked to disease resistance genes, demonstrating the potential of NGS for rapid marker development and QTL mapping (Yang et al., 2012). Similar approaches can be applied to *Camellia* breeding programs to enhance the efficiency of selecting desirable traits.



In summary, the development and application of genetic markers in *Camellia* breeding programs have significantly advanced with the use of molecular techniques. These markers facilitate the identification of genetic diversity, construction of linkage maps, and QTL analysis, ultimately improving the efficiency of marker-assisted selection in *Camellia* breeding.

# 4 Application of Marker-Assisted Selection in Camellia Breeding

#### 4.1 Marker-assisted backcrossing (MABC)

Marker-Assisted Backcrossing (MABC) is a powerful technique used to introduce or enhance specific traits in elite *Camellia* cultivars. This method involves backcrossing a hybrid progeny with one of its parents while using genetic markers to select for the desired trait and the background genome of the elite parent (Kim et al., 2019).

The steps of MABC are to cross the donor parent, which possesses the desired trait, with the recurrent parent, an elite cultivar; to screen the progeny using genetic markers to identify individuals carrying the desired trait; to backcross selected progeny with the recurrent parent; and to continue backcrossing and selecting progeny over several generations to recover the recurrent parent's genome while retaining the desired trait.

### 4.2 Marker-assisted recurrent selection (MARS)

Marker-Assisted Recurrent Selection (MARS) is employed to improve polygenic traits by selecting individuals with the best combination of genetic markers associated with the desired characteristics over multiple generations. The steps of MARS are to cross individuals with desirable traits and screen progeny using multiple genetic markers linked to these traits; to select the best individuals based on marker data and intercross them; and to repeat the selection and intercrossing process over several generations to accumulate favorable alleles (Ali et al., 2020).

#### 4.3 Genomic selection (GS)

Genomic Selection (GS) represents a more advanced approach where genome-wide marker data is used to predict the breeding value of individuals. Unlike MABC and MARS, GS considers the cumulative effect of all genetic markers across the genome, providing a more comprehensive selection strategy. The steps of GS are collecting genotype and phenotype data from a reference population; developing predictive models using statistical methods to associate marker data with phenotypic traits; and using the models to predict the breeding values of new individuals and select the best candidates for breeding (Spindel et al., 2015).

#### 4.4 Case studies and success stories in Camellia breeding

#### 4.4.1 Case study 1: enhancing disease resistance in *Camellia lutchuensis*

Kondratev et al. (2020) used *Ciborinia camellia* to induce rapid infection in the flowers of the susceptible cultivar 'Nicky Crisp' (*Camellia japonica* × *Camellia pitardii* var. *pitardii*), while *Camellia lutchuensis* showed highly resistant. Genome-wide analysis of antagonistic plant gene expression revealed that after 6 hours of inoculation with ascospores, the host transcriptional activity was rapidly regulated (Figure 1). The research highlighted that the early induction of defense responses in *Camellia* plants significantly reduced disease development.

#### 4.4.2 Case study 2: improving cold tolerance in Camellia oleifera

Breeders identified markers associated with cold tolerance and used them to select and intercross the best-performing individuals over several generations. Wu et al. (2020) utilized Illumina NGS technology to perform transcriptomic analyses of *Camellia oleifera* 'Huaxin' leaves under long-term cold stress. This study identified a group of cold-responsive genes related to hormone regulation, photosynthesis, membrane systems, and osmoregulation, which are critical for chilling tolerance. Meanwhile, Wu et al. (2020) measured some physiological indicators of 'Huaxin' *Camellia oleifera* under three temperature conditions, and the results were consistent with molecular sequencing results (Figure 2).

#### 4.4.3 Case study 3: advancing tea quality in Camellia sinensis

Genomic selection has been transformative for improving tea quality in *Camellia sinensis*. By integrating genome-wide marker data with traditional phenotypic evaluations, breeders have significantly accelerated the



selection process for traits such as catechin content, flavor profile, and drought tolerance. Lubanga et al. (2022) stated that genomic selection strategies have been found to increase genetic gain in tea breeding programs (Figure 3), significantly outperforming traditional phenotypic selection methods.

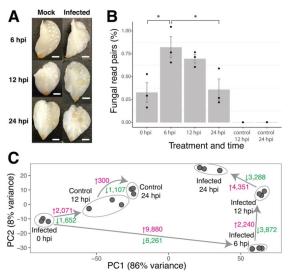


Figure 1 Interactions between *Ciborinia camelliae* and resistant *Camellia lutchuensis* (Adopted from Kondratev et al., 2020) Image caption: A, Development of the resistance response in *C. lutchuensis*. Scale bar = 0.5 cm. B, Proportion of reads mapped to the fungal genome. Each dot represents an observed value, bars represent the average value at each timepoint and treatment, error bars represent standard error. Asterisks (\*) indicate P < 0.05 (two-tailed Student's *t* test). C, Principal component analysis of plant read count data. Axes show first and second principal components (PC1 and PC2, respectively). Each dot represents a biological replicate, circles represent matched biological replicates, arrows indicate the time course, numbers represent upregulated (pink) or downregulated (green) transcripts. hpi = hours postinoculation (Adopted from Kondratev et al., 2020)

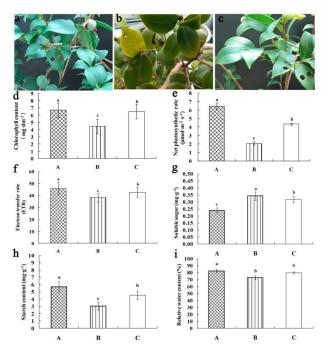


Figure 2 Effect of different temperature treatments on phenotypic changes, chlorophyll contents, net photosynthetic rate (Pn), electron transfer rate (ETR), soluble sugar, starch content, and relative water contentin *Camellia oleifera* 'Huanxin' leaves (Adopted from Wu et al., 2020)

Image caption: (a) 25 °C; (b) 6 °C; (c) Low environmental temperature; (d) Chlorophyll content; (e) Net photosynthetic rate; (f) Electron transfer rate; (g) Soluble sugar content; (h) Starch content; (i) Relative water content. Data represent the mean  $\pm$  SE (n = 3). Lowercase letters indicate significant differences among different temperature treatments at  $p \le 0.05$  according to Duncan's multiple range test (DMRT) (Adopted from Wu et al., 2020)



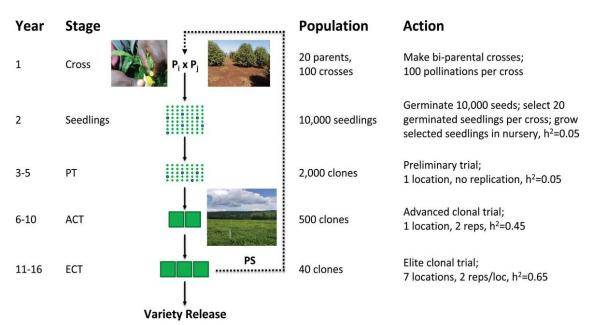


Figure 3 Schematic overview of the phenotypic selection breeding program (PS) (Adopted from Lubanga et al., 2022) Image caption: the dashed line represents the stage at which the 5 or 20 new parents are selected based on phenotypic information. PT, Preliminary Trial stage; ACT, Advanced Clonal Trial stage and ECT, Elite Clonal Trial stage (Adopted from Lubanga et al., 2022)

These case studies underscore the impact of MAS in overcoming traditional breeding challenges and achieving significant advancements in *Camellia* breeding programs. By leveraging the power of genetic markers, breeders can continue to develop innovative and high-performing *Camellia* varieties that meet the demands of both ornamental horticulture and the tea industry.

# **5** Advances in Genomic Technologies and Their Impact on MAS

## 5.1 Next-generation sequencing (NGS) and genotyping-by-sequencing (GBS)

Next-Generation Sequencing (NGS) technologies have revolutionized the field of genomics by providing high-throughput, cost-effective, and accurate sequencing of DNA. Genotyping-by-Sequencing (GBS) is a specific application of NGS that allows for the rapid and simultaneous discovery and genotyping of single nucleotide polymorphisms (SNPs). These technologies enable the identification of thousands of genetic markers across the genome, providing high-resolution genetic maps for MAS (Abebe, 2019). Furthermore, NGS and GBS significantly reduce the cost and time required for genotyping compared to traditional methods, facilitating the precise mapping of quantitative trait loci (QTL) and the identification of markers closely linked to desirable traits.

In *Camellia sinensis*, NGS and GBS have been employed to identify SNPs associated with important traits such as tea quality and disease resistance. These markers are now being used in MAS programs to select for superior tea cultivars. The dense marker coverage provided by these technologies enhances trait mapping and enables breeders to make more informed selections, ultimately improving the efficiency and effectiveness of *Camellia* breeding programs.

## 5.2 Genome-wide association studies (GWAS)

Genome-Wide Association Studies (GWAS) involve scanning the entire genome of a population to identify genetic variants associated with specific traits. GWAS relies on the high-density marker data generated by Next-Generation Sequencing (NGS) and Genotyping-by-Sequencing (GBS). This approach provides detailed insights into the genetic basis of complex traits by identifying multiple loci that contribute to trait variation, enabling the discovery of novel genetic markers associated with economically important traits, which can be directly used in Marker-Assisted Selection (MAS). By utilizing markers identified through GWAS, breeders can achieve higher selection accuracy and genetic gain (Luo et al., 2023).



In *Camellia japonica*, GWAS has been conducted to identify genetic loci associated with ornamental traits such as flower color and shape. These findings are being integrated into MAS programs to develop new ornamental varieties with enhanced aesthetic qualities. This integration demonstrates the significant impact of GWAS on MAS, facilitating the development of *Camellia* varieties that meet specific breeding objectives and consumer preferences.

### 5.3 CRISPR/Cas9 and gene editing technologies

CRISPR/Cas9 and other gene editing technologies have introduced new possibilities for precise genetic modifications. These tools allow for targeted alterations of specific genes, enabling the creation of desired traits with high precision. CRISPR/Cas9 allows for the precise modification of target genes, which can be combined with MAS to accelerate the breeding of desired traits (Li et al., 2023). Additionally, gene editing can be used to validate the function of candidate genes identified through MAS, enhancing our understanding of trait genetics. These technologies also enable the introduction of novel traits that may not be present in the existing gene pool, expanding the possibilities for breeding.

In *Camellia sinensis*, CRISPR/Cas9 has been used to edit genes related to caffeine synthesis, potentially leading to the development of low-caffeine tea varieties. This application showcases the potential of gene editing to complement MAS in achieving breeding objectives. By utilizing CRISPR/Cas9, breeders can create precise genetic changes that enhance the efficiency and effectiveness of MAS, paving the way for the development of innovative *Camellia* varieties that meet specific breeding goals.

#### 5.4 Integration of genomic data with MAS

The integration of genomic data with MAS involves combining high-throughput genotyping, phenotyping, and bioinformatics tools to enhance breeding efficiency and effectiveness. By integrating genomic data, breeders can develop prediction models that estimate the genetic value of individuals, improving selection accuracy. Advanced bioinformatics tools facilitate the management and analysis of large genomic datasets, enabling the identification of key markers and genes (Sorrells, 2015). This integration also allows for the implementation of holistic breeding strategies that consider multiple factors influencing trait expression.

In *Camellia oleifera*, the integration of genomic data with MAS has led to the development of improved oil-yielding varieties. By using genomic prediction models, breeders have been able to select individuals with higher oil content and better disease resistance, resulting in more efficient and targeted breeding programs. These advancements demonstrate the significant potential of integrating genomic data with MAS to drive the development of superior *Camellia* varieties.

## 6 Challenges and Future Prospects in MAS for Camellia

#### 6.1 Technical and economic challenges

Marker-assisted selection (MAS) in *Camellia* breeding programs faces several technical and economic challenges. One significant technical challenge is the development and validation of reliable molecular markers that are closely linked to traits of interest. For instance, in the study on *Camellia oleifera*, Sequence-Related Amplified Polymorphism (SRAP) markers were used to guide hybridization and selection, but the process required extensive validation to ensure accuracy and reliability (Feng et al., 2020). Additionally, the integration of MAS with conventional breeding methods can be complex and resource-intensive, requiring substantial investment in both technology and expertise (Jena and Mackill, 2008; Miedaner and Korzun, 2012).

Economic challenges also play a crucial role in the implementation of MAS. The initial costs of developing and validating markers, as well as the ongoing expenses associated with high-throughput genotyping, can be prohibitive for many breeding programs (Gupta et al., 2010). Furthermore, the return on investment may be slow, particularly for crops like *Camellia*, where the breeding cycle is long, and the market demand for new varieties may not be as high as for staple crops like wheat or rice (Miedaner and Korzun, 2012).



#### 6.2 Ethical and regulatory considerations

Ethical and regulatory considerations are increasingly important in the application of MAS in plant breeding. One ethical concern is the potential for reduced genetic diversity due to the selection of specific traits, which could make crops more vulnerable to diseases and environmental changes (Yang et al., 2008; Feng et al., 2020). Ensuring that breeding programs maintain a broad genetic base is essential for the long-term sustainability of *Camellia* species.

Regulatory frameworks also impact the adoption of MAS. Different countries have varying regulations regarding the use of genetically modified organisms (GMOs) and the release of new plant varieties. While MAS itself does not involve genetic modification, the regulatory landscape can still influence its adoption due to the close association with biotechnological methods. Compliance with these regulations can add to the complexity and cost of breeding programs, potentially slowing down the development and release of new *Camellia* varieties.

#### 6.3 Future directions and potential research areas

The future of MAS in *Camellia* breeding programs holds several promising directions and potential research areas. One key area is the development of more efficient and cost-effective genotyping technologies. Advances in high-throughput sequencing and the use of single nucleotide polymorphisms (SNPs) could significantly reduce the costs and increase the precision of MAS (Gupta et al., 2010; Hasan et al., 2021).

Another promising direction is the integration of genomic selection (GS) with MAS. GS uses genome-wide markers to predict the breeding value of individuals, which can accelerate the selection process and improve the accuracy of breeding programs (Jena and Mackill, 2008; Miedaner and Korzun, 2012). Combining GS with MAS could enhance the efficiency of selecting for complex traits, such as yield and stress resistance, in *Camellia*.

Research into the genetic basis of important traits in *Camellia*, such as disease resistance and oil content, is also crucial. Identifying and mapping quantitative trait loci (QTLs) associated with these traits can provide valuable markers for MAS and help breeders develop more resilient and productive varieties (Feng et al., 2020). Additionally, exploring the potential of gene pyramiding, where multiple genes for a single trait are combined, could offer new opportunities for improving *Camellia* breeding outcomes (Jena and Mackill, 2008).

In conclusion, while there are significant challenges to the implementation of MAS in *Camellia* breeding programs, ongoing research and technological advancements hold great promise for overcoming these obstacles and enhancing the efficiency and effectiveness of breeding efforts.

#### 7 Concluding Remarks

This study has highlighted the significant advancements and applications of marker-assisted selection (MAS) in *Camellia* breeding programs. Key findings include the extensive genetic diversity and breeding objectives within the genus *Camellia*, which encompasses over 250 species with significant economic and ornamental value. Breeding efforts focus on enhancing traits such as flower color and shape, disease resistance, cold tolerance, and oil content and quality. Various genetic markers, including morphological, biochemical, and molecular markers (RFLP, AFLP, SSR, SNP), are employed in *Camellia* breeding, with molecular markers offering high precision and reliability for genetic analysis. The development of genetic markers has been crucial in creating linkage maps and identifying quantitative trait loci (QTL) associated with desirable traits in *Camellia* species. Additionally, MAS strategies such as Marker-Assisted Backcrossing (MABC), Marker-Assisted Recurrent Selection (MARS), and Genomic Selection (GS) have significantly improved the efficiency and precision of *Camellia* breeding programs.

The findings from this study have several implications for *Camellia* breeding programs. The use of marker-assisted selection (MAS) allows for more efficient selection of desirable traits, reducing the time and cost associated with traditional breeding methods. Molecular markers enable precise identification and incorporation of traits, ensuring the development of superior *Camellia* varieties with improved performance and quality. Breeding programs can leverage MAS to develop *Camellia* varieties that are resilient to climate change, ensuring



sustainability and productivity in varying environmental conditions. Additionally, by focusing on key traits such as flower aesthetics, disease resistance, and oil quality, MAS can significantly enhance the commercial and ornamental value of *Camellia* species.

Marker-assisted selection has emerged as a powerful tool in *Camellia* breeding, offering numerous advantages over traditional methods. To fully harness the potential of MAS, several recommendations are proposed. Continued investment in genomic resources, including sequencing and annotating *Camellia* genomes, developing high-density genetic maps, and expanding marker databases, is essential to support MAS. Integrating advanced technologies such as CRISPR/Cas9, genomic selection, and high-throughput phenotyping with MAS will enhance breeding outcomes. Promoting collaboration between public research institutions, private breeding companies, and international organizations will facilitate the sharing of resources, expertise, and innovative approaches. Engaging with the public and stakeholders to communicate the benefits and safety of MAS-derived *Camellia* varieties will foster acceptance and support for these technologies. Breeding programs should also prioritize developing *Camellia* varieties that contribute to sustainable agriculture, focusing on traits related to resource use efficiency, climate resilience, and environmental impact.

In conclusion, the application of MAS in *Camellia* breeding represents a transformative approach that can drive the development of superior varieties, meeting the demands of both ornamental horticulture and commercial production. By addressing current challenges and embracing future research directions, breeders can unlock the full potential of MAS, ensuring the continued success and sustainability of *Camellia* breeding programs.

#### Acknowledgments

The authors extend sincere thanks to two anonymous peer reviewers for their feedback on the manuscript.

#### **Conflict of Interest Disclosure**

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

#### References

- Abebe K., 2019, Genotype by sequencing method and its application for crop improvement (a review), Archives of Biochemistry and Biophysics, 7(1): 1-7. https://doi.org/10.11648/j.abb.20190701.11
- Ali M., Zhang L., DeLacy I., Arief V., Dieters M., Pfeiffer W., Wang J., and Li H., 2020, Modeling and simulation of recurrent phenotypic and genomic selections in plant breeding under the presence of epistasis, Crop Journal, 8(5): 866-877. https://doi.org/10.1016/j.cj.2020.04.002
- Bali S., Raina S., Bhat V., Aggarwal R., and Goel S., 2013, Development of a set of genomic microsatellite markers in tea (*Camellia* L.) (Camelliaceae). Molecular Breeding, 32: 735-741. <u>https://doi.org/10.1007/s11032-013-9902-4</u>
- Chang Y., Oh E., Lee M., Kim H., Moon D., and Song K., 2017, Construction of a genetic linkage map based on RAPD, AFLP, and SSR markers for tea plant (*Camellia sinensis*), Euphytica, 213: 190. https://doi.org/10.1007/s10681-017-1979-0

Dubey H., Rawal H., Rohilla M., Lama U., Kumar P., Bandyopadhyay T., Gogoi M., Singh N., and Mondal T., 2020, TeaMiD: a comprehensive database of simple sequence repeat markers of tea, Database, 2020: baaa013. <u>https://doi.org/10.1093/database/baaa013</u>

PMid:32159215 PMCid:PMC7065459

Feng J., Jiang Y., Yang Z., Chen S., El-Kassaby Y., and Chen H., 2020, Marker-assisted selection in *C. oleifera* hybrid population, Silvae Genetica, 69: 63-72. <u>https://doi.org/10.1093/database/baaa013</u> PMid:32159215 PMCid:PMC7065459

Foolad M., and Panthee D., 2012, Marker-assisted selection in tomato breeding, Critical Reviews in Plant Sciences, 31(2): 93-123. https://doi.org/10.1080/07352689.2011.616057

Guo A.Q., Feng H.F., Jing P., Lan Y., and Cao X.N., 2024, White tea: a review on composition characteristics, extraction techniques, and application potentials, Journal of Tea Science Research, 14(1): 19-43. https://doi.org/10.5376/itsr.2024.14.0003



- Gupta P., Langridge P., and Mir R., 2010, Marker-assisted wheat breeding: present status and future possibilities, Molecular Breeding, 26: 145-161. https://doi.org/10.1007/s11032-009-9359-7
- Hasan N., Choudhary S., Naaz N., Sharma N., and Laskar R., 2021, Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes, Journal of Genetic Engineering & Biotechnology, 19(1): 128.

https://doi.org/10.1186/s43141-021-00231-1

PMid:34448979 PMCid:PMC8397809

- Jena K., and Mackill D., 2008, Molecular markers and their use in marker-assisted selection in rice, Crop Science, 48: 1266-1276. https://doi.org/10.2135/cropsci2008.02.0082
- Jiang M.G., Wei S.Z., and Zhou Y., 2024, Development of novel fermented tea products through microbial community engineering, Journal of Tea Science Research, 14(1): 44-51.

https://doi.org/10.5376/jtsr.2024.14.0004

Karunarathna K., Mewan K., Weerasena O., Perera S., and Edirisinghe E., 2020, A functional molecular marker for detecting blister blight disease resistance in tea (*Camellia sinensis* L.), Plant Cell Reports, 40: 351-359.

https://doi.org/10.1007/s00299-020-02637-6 PMid:33247387

- Kim J., Jung Y., Seo H., Kim M., Nou I., and Kang K., 2019, Recurrent parent genome (RPG) recovery analysis in a marker-assisted backcross breeding based on the genotyping-by-sequencing in tomato (*Solanum lycopersicum* L.), Journal of Plant Biotechnology, 46(3): 165-171. <u>https://doi.org/10.5010/JPB.2019.46.3.165</u>
- Kondratev N., Denton-Giles M., Bradshaw R., Cox M., and Dijkwel P., 2020, Camellia plant resistance and susceptibility to petal blight disease are defined by the timing of defence responses, Molecular Plant-Microbe Interactions, 33(7): 872-1006. <u>https://doi.org/10.1094/MPMI-10-19-0304-R</u>

PMid:32223579

Li H., Song K., Li B., Zhang X., Wang D., Dong S., and Yang L., 2023, CRISPR/Cas9 editing sites identification and multi-elements association analysis in *Camellia sinensis*, International Journal of Molecular Sciences, 24(20): 15317. <u>https://doi.org/10.3390/ijms242015317</u>

PMid:37894996 PMCid:PMC10607008

Li Q., Su X., Ma H., Du K., Yang M., Chen B., Fu S., Fu T., Xiang C., Zhao Q., and Xu L., 2021, Development of genic SSR marker resources from RNA-seq data in *Camellia japonica* and their application in the genus *Camellia*, Scientific Reports, 11: 9919. <u>https://doi.org/10.1038/s41598-021-89350-w</u>

PMid:33972624 PMCid:PMC8110538

- Liu C.C., 2024, Interaction between tea tree root probiotics and tea yellowing disease, Journal of Tea Science Research, 14(1): 10-18. https://doi.org/10.5376/jtsr.2024.14.0002
- Liu S., An Y., Li F., Li S., Liu L., Zhou Q., Zhao S., and Wei C., 2018, Genome-wide identification of simple sequence repeats and development of polymorphic SSR markers for genetic studies in tea plant (*Camellia sinensis*), Molecular Breeding, 38: 59. https://doi.org/10.1007/s11032-018-0824-z
- Lubanga N., Massawe F., Mayes S., Gorjanc G., and Bančič J., 2022, Genomic selection strategies to increase genetic gain in tea breeding programs, The Plant Genome, 16(1): e20282.

https://doi.org/10.1002/tpg2.20282

PMid:36349831

- Luo H., Pan X., Huang Y., Li Z., Ye H., Zhou K., Wen L., Qin J., He W., Du X., Wang D., and Lin Y., 2023, Genome-wide association study reveals markers and candidate genes associated with growth in the rice flower carp, an economic fish species of integrated rice-fish culture in China, Frontiers in Marine Science, 10: 1130667. https://doi.org/10.3389/fmars.2023.1130667
- Ma J., Zhou Y., Ma C., Yao M., Jin J., Wang X., and Chen L., 2010, Identification and characterization of 74 novel polymorphic EST-SSR markers in the tea plant, *Camellia sinensis* (Theaceae), American Journal of Botany, 97(12): e153-e156.

https://doi.org/10.3732/ajb.1000376

Malebe M., Koech R., Mbanjo E., Kamunya S., Myburg A., and Apostolides Z., 2021, Construction of a DArT-seq marker-based genetic linkage map and identification of QTLs for yield in tea (*Camellia sinensis* (L.) O. Kuntze), Tree Genetics & Genomes, 17: 9. <u>https://doi.org/10.1007/s11295-021-01491-1</u>

Miedaner T., and Korzun V., 2012, Marker-assisted selection for disease resistance in wheat and barley breeding, Phytopathology, 102(6): 560-566. https://doi.org/10.1094/PHYTO-05-11-0157

PMid:22568813

Sharma R., Bhardwaj P., Negi R., Mohapatra T., and Ahuja P., 2009, Identification, characterization and utilization of unigene derived microsatellite markers in tea (*Camellia sinensis* L.), BMC Plant Biology, 9: 53.

https://doi.org/10.1186/1471-2229-9-53

PMid:19426565 PMCid:PMC2693106



- Sorrells M., 2015, Genomic selection in plants: empirical results and implications for wheat breeding, In: Ogihara Y., Takumi S., Handa H. (eds.), Advances in wheat genetics: from genome to field, Springer, Tokyo, Japan, pp.401-409. https://doi.org/10.1007/978-4-431-55675-6\_45
- Spindel J., Begum H., Akdemir D., Virk P., Collard B., Redoña E., Atlin G., Jannink J., and McCouch S., 2015, Genomic selection and association mapping in rice (*Oryza sativa*): effect of trait genetic architecture, training population composition, marker number and statistical model on accuracy of rice genomic selection in elite, tropical rice breeding lines, PLoS Genetics, 11(6): e1005350. <u>https://doi.org/10.1371/journal.pgen.1005350</u>

PMid:26125618 PMCid:PMC4488320

Tan L., Wang L., Wei K., Zhang C., Wu L., Qi G., Cheng H., Zhang Q., Cui Q., and Liang J., 2013, Floral transcriptome sequencing for SSR marker development and linkage map construction in the tea plant (*Camellia sinensis*), PLoS One, 8(11): e81611.
 <u>https://doi.org/10.1371/journal.pone.0081611</u>
 PMid:24303059 PMCid:PMC3841144

Tian Q., Huang B., Huang J., Wang B., Dong L., Yin X., Gong C., and Wen Q., 2022, Microsatellite analysis and polymorphic marker development based on the full-length transcriptome of *Camellia chekiangoleosa*, Scientific Reports, 12: 18906. <u>https://doi.org/10.1038/s41598-022-23333-3</u> PMid:36344600 PMCid:PMC9640616

- Wang X., Zheng H.Y., Zheng W.H., Ao C.Q., Jin H.Y., Zhao L.H., Li N., and Jia L.R., 2011, RAPD-based genetic diversities and correlation with morphological traits in *Camellia* (Theaceae) cultivars in China, Genetics and Molecular Research, 10(2): 849-859. <u>https://doi.org/10.4238/vol10-2gmr1207</u> PMid:21574141
- Wu L., Li J., Li Z., Zhang F., and Tan X., 2020, Transcriptomic analyses of *Camellia oleifera* 'Huaxin' leaf reveal candidate genes related to long-term cold stress, International Journal of Molecular Sciences, 21(3): 846.
  <u>https://doi.org/10.3390/ijms21030846</u>
  PMid:32013013 PMCid:PMC7037897
- Yan H., Qi H., Li Y., Wu Y., Wang Y., Chen J., and Yu J., 2022, Assessment of the genetic relationship and population structure in oil-tea *Camellia* species using simple sequence repeat (SSR) markers, Genes, 13(11): 2162.
  <u>https://doi.org/10.3390/genes13112162</u>
  PMid:36421835 PMCid:PMC9691144
- Yang H., Renshaw D., Thomas G., Buirchell B., and Sweetingham M., 2008, A strategy to develop molecular markers applicable to a wide range of crosses for marker assisted selection in plant breeding: a case study on anthracnose disease resistance in lupin (*Lupinus angustifolius* L.), Molecular Breeding, 21: 473-483.

https://doi.org/10.1007/s11032-007-9146-2

Yang H., Tao Y., Zheng Z., Li C., Sweetingham M., and Howieson J., 2012, Application of next-generation sequencing for rapid marker development in molecular plant breeding: a case study on anthracnose disease resistance in *Lupinus angustifolius* L., BMC Genomics, 13: 318. <u>https://doi.org/10.1186/1471-2164-13-318</u>

PMid:22805587 PMCid:PMC3430595

- Yao X.Z., Tang H., Jiao Y.J., He Y.M., and Lu L.T., 2024, Genomic insights into the evolutionary history of the Camellia genus: comprehensive analysis of phylogenetic relationships, speciation, and adaptive evolution, Journal of Tea Science Research, 14(1): 64-78. https://doi.org/10.5376/jtsr.2024.14.0007
- Zhang J., Huang B.F., and Xu G.M., 2024, Caffeine degradation pathways mediated by microbial communities in tea fermentation, Journal of Tea Science Research, 14(1): 57-63.

https://doi.org/10.5376/jtsr.2024.14.0006



#### **Disclaimer/Publisher's Note**

The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and do not represent the views of the publishing house and/or its editors. The publisher and/or its editors disclaim all responsibility for any harm or damage to persons or property that may result from the application of ideas, methods, instructions, or products discussed in the content. Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.