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# The Selection and Evaluation of Excellent Varieties of Tongzi Yimucao Based on Molecular Marker Technology

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**Abstract** This study explored how to improve the variety of Tongzi Yimucao with molecular labeling technology and proposed methods to solve the problems encountered in the breeding process. Research has found that molecular markers such as SSR, SNP, and RAPD can provide accurate genetic maps, which are useful for breeders to select plants with good traits such as high medicinal components and strong disease resistance. MAS can make the breeding process faster and more accurate. Genomic technology is beneficial for constructing a clearer genetic map and also makes it easier to identify genes related to important traits. This study demonstrates that the combination of modern genetic technology and traditional breeding experience is helpful in cultivating high-quality, long-term plant-suitable Tongzi Yimucao varieties to meet the current demand for traditional Chinese medicinal materials.

**Keywords** Tongzi Yimucao; Molecular marker-assisted selection (MAS); Genomic technologies; Medicinal traits; Breeding efficiency

## 1 Introduction

Tongzi Yimucao is a very common herb in traditional Chinese medicine and is widely used in gynecology. It can promote blood circulation, regulate menstruation and relieve pain. As a native medicinal plant, the cultivation and use of Tongzi Yimucao have a long history in traditional Chinese medicine and are suitable for treating menstrual disorders and postpartum recovery, etc. (Zhao, 2009; Wang, 2024).

Tongzi Yimucao has therapeutic effects because it contains many active natural components such as flavonoids, alkaloids and saponins. These components have anti-inflammatory, antioxidant and analgesic effects, so they can be used to help treat many diseases. Qian et al. (2020) and Duan et al. (2022) found that flavonoids are beneficial to the cardiovascular system and the female reproductive system, and saponins have anti-tumor and antibacterial effects.

This study will elaborate on the application of MAS in improving the varieties of Tongzi Yimucao, discuss the solutions to the problems encountered during the cultivation process, and hope to find and cultivate varieties of Tongzi Yimucao with more effective components, making the medicinal effect more stable and stronger, and retaining its uses in traditional Chinese medicine. This study aims to better meet the current demand for high-quality herbs by combining traditional knowledge with modern genetic techniques.

## 2 Molecular Marker Technology Overview and its Application in Tongzi Yimucao

### 2.1 Definition of molecular markers and their classification

Molecular markers are DNA fragments related to specific locations in the genome and can be used to identify different individuals or species, track the transmission of genetic information, and assist in breeding efforts. Simple sequence duplication (SSR), single nucleotide polymorphism (SNP), and random expanded polymorphic DNA (RAPD) are three common molecular markers (Agarwal et al., 2008; Duran et al., 2009; Grover and Sharma, 2016). SSR is a series of repetitive short DNA sequences with large variations, large quantities and wide distribution, and is commonly used in genetic research (Duran et al., 2009; Kocaman et al., 2020). The early studies of Semagn et al. (2006) and Duran et al. (2009) indicated that SNP is a situation where a base in a DNA sequence changes. It is the most common type of variation and is often used for high-resolution mapping and trait association studies.

RAPD marks are to amplify random DNA fragments in the genome by PCR using a random short primer. It is simple to operate and has a low cost, but sometimes the repeatability is not very good (Semagn et al., 2006; Agarwal et al., 2008). Other common ones include AFLP and ISSR. AFLP combines the stability of RFLP and the flexibility of PCR, making it suitable for high-accuracy detection. ISSR is used to detect polymorphisms between microsatellite regions (Agarwal et al., 2008; Kocaman et al., 2020). Grover and Sharma (2016) hold that each marker has its own advantages and disadvantages. The choice of which marker to use depends on the specific research needs such as the degree of polymorphism detected, experimental cost and ease of operation.

### 2.2 Application and role of molecular marker-assisted selection (MAS) in Tongzi Yimucao breeding

MAS can directly identify the varieties of Tongzi Yimucao with high medicinal components or strong disease resistance and other good traits at the DNA level. Traditional breeding methods require waiting for plants to grow out and then judging whether they have good traits based on their appearance features, which takes a long time. Kumar (1999) and Soriano’s research in 2020 found that the medicinal effects of Tongzi Yimucao are related to some specific genes, and these traits are not very obvious in the early stage of plant growth. Applying the MAS method to the breeding of Tongzi Yimucao can enable breeders to select high-quality varieties with consistent medicinal components more quickly and accurately, and ensure that the bred plants meet the expected medicinal standards (Kumar, 1999).

Traits such as disease resistance and stable yield are crucial in the breeding of Tongzi Yimucao. MAS can identify the genes related to these traits more quickly and improve the breeding efficiency (Kumar, 1999), and is also helpful for breeders to select plants that have stronger resistance to pests, diseases and environmental changes. The varieties bred will be more stable and have more reliable medicinal effects. Soriano (2020) found that MAS enables brewers to more accurately select strong and high-yielding Tongzi Yimucao varieties in today's increasingly severe climate change.

### 2.3 Key applications of molecular marker technology in enhancing disease resistance, medicinal traits, and yield in Tongzi Yimucao

Tongzi Yimucao is often infected by fungi or bacteria, which affects its yield and efficacy. Breeders can make it easier to pick out plants with stronger disease resistance by finding molecular markers related to disease-resistant genes. They can also use less pesticides, making the cultivation more environmentally friendly and sustainable. The use of these markers can also make the breeding process faster and identify which plants have disease-resistant genes at an early stage. Kumar (1999) and Soriano (2020) hold that this enables plants to grow healthier and more stably, enhancing the stability of yield and medicinal effects.

The medicinal effect of Tongzi Yimucao mainly comes from key active components such as flavonoids and phenolic acids, which have a significant impact on the therapeutic effect. Breeders can more easily pick out plants with higher contents of active ingredients by using genetic markers related to the synthesis of these substances. This method can specifically improve and enhance the medicinal quality, making the bred varieties more effective (Kumar, 1999; Soriano, 2020). These practices are beneficial for maintaining the medicinal properties of Tongzi Yimucao and better meeting the needs of traditional Chinese medicine (Kumar, 1999; Kocaman et al., 2020).

## 3 Molecular Marker Techniques for Plant Breeding

### 3.1 Detailed discussion of various molecular marker techniques

Molecular marker techniques such as SSR, SNP and AFLP are mainly used in the breeding process of Tongzi Yimucao to evaluate genetic diversity, locate traits and conduct molecular marker-assisted selection. Maibody et al. (2019) and Benchimol-Reis (2023) demonstrated that SSR markers have high polymorphism and are particularly suitable for Tongzi Yimucao, enabling more accurate identification of plants with rich genetic backgrounds. Researchers often use these markers to analyze the differences among different populations, facilitating the identification of good varieties with high medicinal components or strong disease resistance. Yang et al. (2015) and Maibody et al. (2019) found that SSR is also helpful for constructing genetic maps, which can help to know more clearly which genes are related to superior traits and improve new varieties with obvious target traits more quickly.

SNP markers can identify small genetic differences related to the content of medicinal components or disease resistance in the breeding of Tongzi Yimucao, allowing breeders to select plants more accurately based on these key traits and enhance the medicinal effect. Li et al. (2001) found in earlier studies and Sharma and Sharma in 2018 that AFLP markers are generally used in the early stage of breeding or as a substitute when other markers are not effective. AFLP is particularly sensitive in discovering genetic differences within a population and is useful for understanding the genetic structure of a population and identifying parents that are promising to breed good offspring. Maibody et al. (2019) and Benchimol-Reis (2023) hold that the combined use of these molecular techniques enables breeders to obtain more comprehensive and accurate genetic information and improve the efficiency of the breeding process.

### 3.2 Advantages and disadvantages of different types of markers in Tongzi Yimucao breeding

The SSR marker has high polymorphism and can be co-dominant inherited. It is applicable to many different plant species and is very popular. Maibody et al. (2019) and Benchimol-Reis (2023) found that it can distinguish homozygous and heterozygous genotypes and is suitable for selecting plants with high disease resistance or medicinal component content. However, it takes a considerable amount of time to make primers for SSR, and the cost is not low either. Therefore, the cost of using it will be relatively high. However, SSR markers still play an important role in many breeding projects because they are reliable and can be used for multiple purposes. They are widely used in conducting large-scale diversity studies or drawing genetic maps (Yang et al., 2015; Maibody et al., 2019).

In the breeding of Tongzi Yimucao, the use of SNP markers requires relatively comprehensive genomic information to identify SNP loci related to the target traits. If the genomic information is not complete or has not been fully sequenced, the use of SNPS will be restricted. Fortunately, SNP markers have become more accessible as sequencing technology becomes increasingly advanced, and they will be of greater use when high-precision selection is required. Li et al. (2001) and Sharma and Sharma (2018) demonstrated that AFLP markers can detect genetic differences without prior knowledge of genomic information and are useful at the beginning of breeding or when other markers are not effective. However, AFLP has poor specificity and less repeatability than SSR or SNP. Its application in MAS is not very suitable for long-term use. However, Maibody et al. (2019) and Benchimol-Reis (2023) hold that AFLP can still provide valuable reference information in the early breeding stage, especially when no other suitable markers are available.

### 3.3 The role of genomic technologies in improving the efficiency of molecular marker-assisted selection for medicinal components and disease resistance in Tongzi Yimucao

New technologies such as next-generation sequencing (NGS) and genome-wide association studies (GWAS) have made it easier to identify genes related to key traits. Yang et al. (2015) and Salgotra and Stewart (2020) more accurately located the genes affecting the medicinal components through sequencing and transcriptome analysis of the entire genome, enabling faster and more accurate trait recognition, and making it more certain to select plants containing high levels of flavonoids or phenolic acids to enhance the medicinal effect. Maibody et al. (2019) and Benchimol-Reis (2023) demonstrated in their research that these genomic tools can provide a large amount of genetic information and be linked to specific traits, making the process of MAS more accurate and time-saving.

Jiang (2013) believes that with the improvement of genomic sequencing technology, breeders have been able to identify those locations related to disease resistance, pick out more disease-resistant plants, and prevent fungal or bacterial infections, as these pathogens can reduce yields and affect the efficacy of drugs. Salgotra and Stewart (2020), as well as Karakaş (2024), discovered that when genomic technology is used in combination with molecular markers, disease-resistant genes can be identified more quickly and accurately, leading to the breeding of new varieties of Tongzi Yimucao that are stronger and have higher yields (Figure 1).

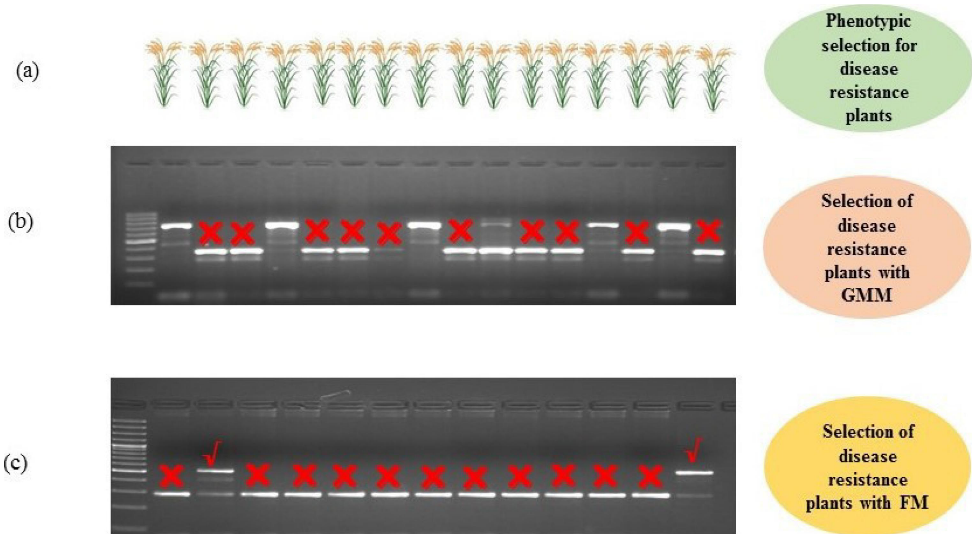

Figure 1 Use of functional markers (FMs) in marker assisted selection in precision breeding (Adopted from Salgotra and Stewart, 2020)

Image caption: (a) Phenotypic selection for disease resistance plants. (b) Selection of disease resistance using genic molecular markers (GMM). (c) Disease resistance plants selection using functional marker (FM) (Adopted from Salgotra and Stewart, 2020)

## 4 Genetic Diversity of Tongzi Yimucao

### 4.1 The genetic diversity within Tongzi Yimucao populations and its significance in breeding programs

The genetic diversity of the Tongzi Yimucao population is helpful for plants to adapt to various environmental conditions and for species to survive for a long time. Scientists have found significant genetic differences in the growth rate, content of active ingredients, disease resistance and stress tolerance of Tongzi Yimucao. Zhao's research in 2009 suggested that these differences were very helpful for breeding efforts, as they provided abundant genetic resources that could be used to cultivate new species with stronger medicinal effects.

Genetic diversity is a basis for selecting superior traits and cultivating ideal hybrid varieties. Breeders rely on these genetic differences to address various challenges such as climate change, pests and diseases, and the market demand for varieties with high medicinal value. Song and Li's research in 2020 demonstrated that a population with rich genetic diversity can accelerate the breeding speed of good varieties, avoid the problem of genetic bottlenecks, and reduce the impact on the stable growth and yield of Tongzi Yimucao.

### 4.2 Methods for evaluating genetic diversity using molecular markers

Technologies such as RAPD, SSR and ISSR can directly detect the differences among plants at the DNA level. These methods enable researchers to identify polymorphisms among different groups, which is helpful for constructing genetic maps and understanding the distribution of various traits in the population. This information is crucial for finding materials with unique genetic resources and formulating more scientific breeding strategies.

High-throughput technologies such as GWAS and NGS can bring more genetic information, which is helpful for researchers to have a clearer understanding of the genetic structure of a population and to more easily identify genes related to medicinal traits. Qian et al. 's research in 2020 demonstrated that this data-driven approach can enhance the accuracy of molecular marker-assisted selection, giving breeders more confidence in developing high-quality new varieties of Tongzi Yimucao that meet specific medicinal needs.

### 4.3 The role of genetic diversity in enhancing the adaptability and resilience of cultivated varieties

Groups with relatively rich genetic variations are usually more adaptable to environmental factors such as temperature changes, variations in the amount of water resources, and soil differences. Tongzi Yimucao is often planted in different ecological areas, and this adaptability is very important for its cultivation. Maintaining genetic diversity enables the cultivated varieties to grow well under various climatic conditions and be less susceptible to environmental stress.

If a variety has a broad genetic basis, it usually carries multiple resistance genes and is not easily defeated by pests or pathogens at once. This stress resistance can ensure stable yields and reduce the use of chemicals such as pesticides, making it more environmentally friendly and more in line with the concept of sustainable agriculture. The early research by Sarwat et al. (2012) indicated that breeders could possibly select and breed new varieties of Tongzi Yimucao with high yield, disease resistance and stress tolerance by taking advantage of the rich genetic diversity, in order to address various challenges in the cultivation of medicinal plants.

## 5 Evaluation of Medicinal Traits in Tongzi Yimucao

### 5.1 Identification of key medicinal traits using molecular markers

Researchers can identify quantitative trait loci (QTL) related to bioactive components through molecular markers, and these components directly affect the efficacy of traditional Chinese medicinal plants such as Tongzi Yimucao. Song, Li and Qian et al. found in their research in the same year, that is, in 2020, that the use of MAS can purposefully increase the content of these active ingredients and improve the overall quality and efficacy of medicinal plants. This method can accelerate breeding and ensure that the selected varieties have better medicinal properties.

To identify the highly active components in Tongzi Yimucao through molecular markers, researchers need to conduct a comprehensive analysis of its genetic composition. With the help of molecular marker techniques such as SNP, genetic variations related to the high content of medicinal components can be precisely identified (Figure 2) (Lande and Thompson, 1990; Hasan et al., 2021). This genetic information is very important for cultivating new plant varieties with stronger medicinal effects and can also enable these medicinal traits to be more effectively applied in clinical treatment.

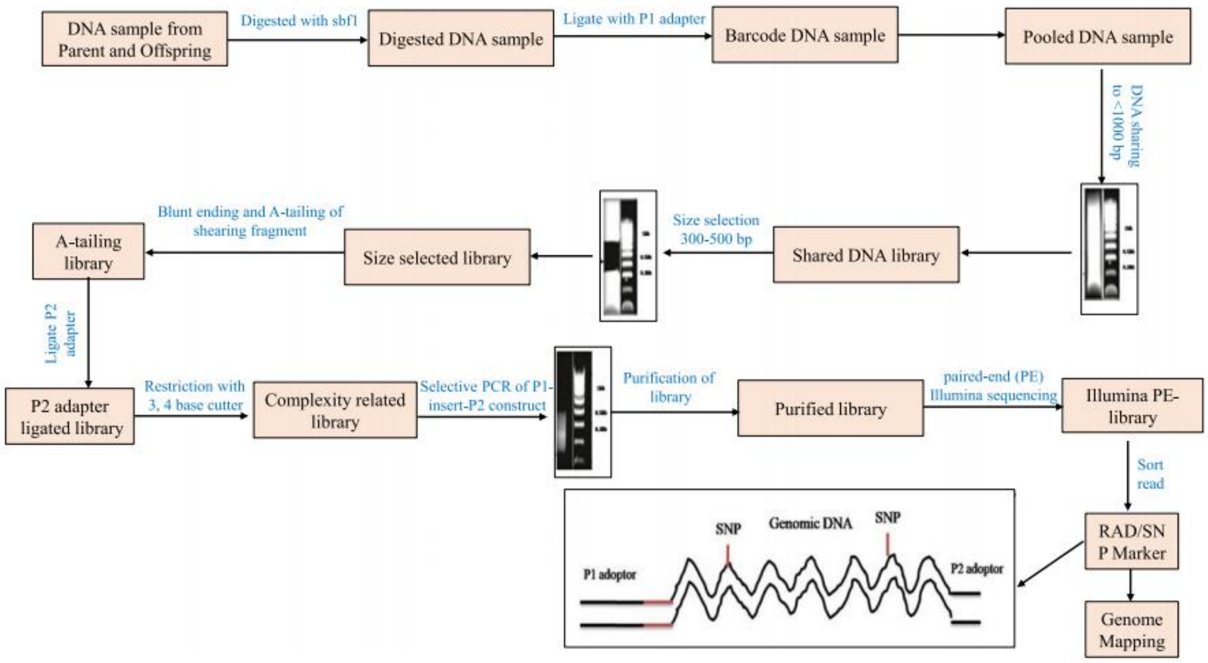

Figure 2 RAD-sequence: discovery and genotyping of SNPs by next-generation sequencing for genome mapping (Adopted from Hasan et al., 2021)

Image caption: EcoRI-MspI ligation as “adapter_P1-EcoRI” and “adapter_P2-MspI” (Adopted from Hasan et al., 2021)

### 5.2 Genetic factors influencing medicinal quality in Tongzi Yimucao

The genetic factors influencing the medicinal quality of Tongzi Yimucao are mainly related to its own genetic diversity and the presence or absence of specific QTLS associated with medicinal traits. The research conducted by Bernardo in 2008 and by Schultz et al. in 2021 found that the differences in the content of active ingredients among different varieties are largely related to genetic diversity. Researchers can identify the genetic causes of these differences by analyzing these genetic markers.

The combination of molecular genetics methods and traditional breeding means can more accurately identify the genetic traits that can improve the quality of medicinal use. El-Hack et al. (2018) and Qian et al. (2020) hold that this method usually employs advanced techniques such as GWAS to identify genetic markers related to the ideal medicinal quality. These markers can deeply reveal the genetic structure behind the medicinal traits, which is useful for breeders to develop new varieties of Tongzi Yimucao with better efficacy and stronger therapeutic ability.

### 5.3 Methods for selecting varieties with superior medicinal properties based on genetic analysis

The research conducted by Kumawat et al. (2020) and Hasan et al. (2021) in recent years has shown that MAS is an important means to improve the efficiency and accuracy of breeding. Breeders can focus on genetic markers related to high-content active ingredients, thus enabling them to cultivate new varieties that meet medicinal needs more quickly. This approach saves a great deal of time and resources compared with traditional breeding methods.

Proteomics and integrative pharmacology can also enhance the selection efficiency. The studies of El-Hack et al. (2018) and Li et al. (2018) in the same year both demonstrated that these techniques can help gain a deeper understanding of which molecular mechanisms affect medicinal traits and provide more dimensional data support for breeding decisions. The combined use of genetic analysis and new technologies can select varieties with outstanding medicinal characteristics and ensure their stability and consistency in terms of quality and efficacy.

## 6 Molecular Marker-Assisted Selection for Improving Tongzi Yimucao

### 6.1 The process of using molecular markers to track desirable traits for breeding

MAS identifies and tracks genes with good traits in Tongzi Yimucao through gene markers. This method uses techniques such as RAPD, SSR, and SNP, which can precisely locate these markers, to identify genetic markers related to the target traits. Once these markers are determined, they can be used as “codes” to help quickly screen out the individuals that meet the requirements in the breeding materials (Zhao, 2009).

Even when plants are still in the early stage of development, MAS can select potential individuals based on these genetic markers, accelerate the breeding process, and reduce the reliance on traditional phenotypic assessment, as phenotypes are often affected by the environment such as climate and soil. Breeders can introduce superior traits such as high flavonoid and saponin content, insect resistance and stress resistance into new varieties more effectively through MAS. Song and Li (2020) hold that this method is useful for breeding Tongzi Yimucao varieties with medicinal value and good field performance.

### 6.2 Case studies of successful molecular marker-assisted selection in medicinal plant breeding

Molecular markers can be used in *Panax ginseng* breeding to increase the content of ginsenosides and enhance its stress resistance. The varieties bred in this way have stronger medicinal effects and can adapt to different climatic environments. The same technology has also been applied to *Scutellaria baicalensis*, which has increased the content of flavonoids through MAS, enhanced the medicinal effect and increased the market value.

When MAS is applied to the breeding of Tongzi Yimucao, it can draw on the successful practices of other plants to select traits that can enhance active components or resist pests and diseases. These cases demonstrate that MAS can combine the research results at the molecular level with traditional breeding methods to breed new varieties with stable efficacy and high quality. Qian et al. (2020) hold that in the breeding process of Tongzi Yimucao, combining genetic linkage maps and GWAS and other technologies will make more full use of the genetic diversity in germplasm resources and breed good varieties that are both stress-resistant and have market prospects.

### 6.3 Potential of MAS in accelerating the development of improved Tongzi Yimucao varieties

MAS can shorten the breeding cycle and predict the target traits more accurately, and has great potential in accelerating the breeding speed of superior Tongzi Yimucao varieties. Traditional breeding mainly relies on observing the phenotypes of plants, which takes several years to see results and is also disturbed by external factors such as weather and soil. MAS could select advantageous individuals based on genetic information when plants were still very small, thus eliminating the need for a large number of field experiments.

Applying MAS technology to the breeding of Tongzi Yimucao can accelerate the development of high-quality varieties and make new varieties more adaptable to the constantly changing climate and market demands. It is helpful for breeders to focus on selecting plants with high medicinal components, pest resistance and stress resistance, ensuring that the final bred varieties meet the quality requirements of medicinal plants, and is also conducive to long-term planting and environmental protection. With the continuous update of molecular marker technology, MAS will play an increasingly significant role in the breeding of Tongzi Yimucao, which is expected to promote the breeding of a new generation of traditional Chinese medicine plants with stronger medicinal effects (Sarwat et al., 2012).

## 7 Challenges and Limitations in Molecular Marker-Assisted Breeding

### 7.1 Technical challenges in marker development

The associations between the markers found in the research and the target traits are often not strong enough, resulting in unsatisfactory effects when these markers are used in actual MAS. Breeders find it difficult to use these markers freely in breeding practice because the experimental design is not reasonable enough or the genetic background of the plants themselves is too complex (Young, 1999). To improve the accuracy of these associations, it is necessary to combine genomics and bioinformatics for analysis, but this is a difficult challenge for many breeding projects (Young, 1999; Wang et al., 2024).

Molecular marker prediction is very effective for simple traits controlled by a few genes. However, if the traits are acted on by many genes together, along with environmental influences, the prediction results will become less stable (Babu et al., 2004; Jiang, 2015). This complexity requires the use of more advanced methods such as whole-genome sequencing and whole-genome selection in order to take all possible related genetic factors into account. However, Xu et al. (2012) indicated that these methods cost a lot of money, have high technical requirements, and also put forward higher requirements for data analysis capabilities. Xu et al. (2012) also proposed that in order to truly solve these problems, it becomes very necessary to develop high-density genotyping tools and more accurate trait measurement methods, so as to enhance the reliability and effect of MAS in actual breeding.

### 7.2 Limitations of molecular markers for complex traits

Song and Li (2020) hold that complex traits such as medicinal value are often not controlled by a single gene, but are jointly determined by multiple quantitative trait loci (QTLs), and are also affected by environmental conditions. Because there are many genes involved and the role of each gene may be relatively small, it is very difficult to find a certain marker that can stably and accurately predict these traits. The effects of many QTLs themselves are not significant, making them difficult to be detected in breeding projects and even more challenging to be repeatedly verified through experiments. This also makes the effects of the markers in practical operations more uncertain (Babu et al., 2004).

Molecular markers are also limited by the current insufficient understanding of the genetic basis of these traits. Begna (2020) indicates that the development of genomics has brought about many new discoveries, but it remains a major challenge to break down these complex traits into manageable and operable parts. Combining molecular marker technology with traditional breeding methods and formulating more comprehensive breeding strategies can truly achieve valuable trait improvement (Jiang, 2015).

### 7.3 High costs and expertise requirements

The application of molecular marker-assisted selection in practical operation also involves cost issues and requires professional technical support, which poses a considerable challenge for many breeding projects. The fact that steps such as genotyping, phenotypic measurement, and data analysis are all costly makes molecular technology difficult to be popularized in some projects with limited budgets. These techniques usually also require advanced experimental equipment and well-trained professionals, raising the threshold of use (Ribaut and De Vicente, 2010; Begna, 2020).

Begna’s research in 2020 proposed that for breeders to make good use of molecular tools, they need to understand molecular genetics and master knowledge in multiple aspects such as bioinformatics analysis and breeding strategies. The demand for technical talents is very high, and this gap is even more obvious in regions where educational and training resources are insufficient. Ribaut and De Vicente (2010) believe that it is particularly necessary to carry out relevant training and capacity-building projects now to assist breeders in mastering these techniques and truly apply molecular marker technology in actual breeding.

## 8 Future Directions and Prospects in Tongzi Yimucao Breeding

### 8.1 Emerging technologies for improving Tongzi Yimucao breeding

New technologies such as CRISPR/Cas9 and GWAS are changing the breeding methods of Tongzi Yimucao. CRISPR/Cas9 can be used for precise gene editing. Scientists use it to directly modify genes related to important traits, increase the content of active ingredients, and enhance the disease resistance of plants, etc. Song and Li (2020) hold that CRISPR/Cas9 can accelerate the breeding speed of superior varieties, reduce unnecessary genetic variations, and is suitable for repairing genetic defects or introducing traits that are difficult to achieve in traditional breeding.

GWAS can link phenotypic differences with genomic information and is suitable for studying complex traits such as tolerance to environmental stress and the content of medicinal components. For Tongzi Yimucao, GWAS can help breeders identify important QTLS related to drug efficacy, which can provide a clear direction for the subsequent development of molecular markers and the design of breeding strategies. The combined use of CRISPR/Cas9 and GWAS can form a synergistic approach. GWAS was used to identify important trait control regions, and CRISPR/Cas9 was used to precisely modify these regions. This approach could enhance the efficiency of breeding new varieties of Tongzi Yimucao with good medicinal efficacy and strong adaptability.

### 8.2 The integration of multi-omics approaches (genomics, transcriptomics, metabolomics) to enhance breeding efficiency

The combination of multi-omics methods is driving the breeding of Tongzi Yimucao into a brand-new stage. Genomics can reveal the overall genetic composition of plants and is useful for scientists to identify genes related to key traits. Transcriptomics analyzes the expression of genes at different time points or in different environments to understand the specific roles of these genes in plants. Qian et al. (2020) demonstrated that the combination of the two can enable a clearer understanding of how complex traits such as the synthesis of medicinal components are regulated.

Metabolomics directly links specific phenotypic traits with the biochemical metabolic pathways in the body by analyzing the bioactive components in Tongzi Yimucao. Once metabolic data are combined with genomic information and transcriptome information, breeders can have a more comprehensive understanding and utilization of these traits. Through this integrated approach, breeders can adopt the methods of systems biology to establish models for predicting breeding effects. Sarwat et al. (2012) hold that this global strategy makes breeding more efficient and can also ensure that the final bred Tongzi Yimucao variety remains stable and consistent in medicinal value.

### 8.3 Potential for combining traditional knowledge with modern biotechnology in the future development of Tongzi Yimucao varieties

Traditional Chinese Medicine (TCM) has accumulated hundreds of years of experience in the cultivation, harvesting and application of Tongzi Yimucao, including a profound understanding of key characteristics such as strong medicinal effect and definite therapeutic effect. These experiences can provide directions for modern breeding, such as which characteristics are considered the most valuable clinically or which forms are the most popular. Chen et al. (2011) proposed that integrating these traditional wisdom into modern breeding is beneficial for selecting varieties that are closer to the practical application requirements and enabling newly bred varieties to better conform to the usage habits and cultural background of traditional Chinese medicine.

The specific growth environment and harvest time can be further analyzed through molecular means to identify the genetic basis related to these traits and make targeted improvements. This approach can maintain traditional medicinal efficacy and cultural values, and ensure that new varieties meet the standards of modern scientific research and drug quality. The early research of Zhao (2009) indicates that the combination of traditional and modern methods can enable breeders to better balance innovation and tradition, allowing the improved Tongzi Yimucao to be recognized by the modern medical system, continue to play a role in the field of traditional Chinese medicine, and achieve long-term development and wide application recognition.

## 9 Conclusion

Molecular marker technology provides precise gene mapping and trait screening tools, which are crucial in the selection and breeding evaluation of Tongzi Yimucao. It can help researchers identify genes related to superior traits, such as those that can increase the content of active ingredients associated with the medicinal effects of Tongzi Yimucao. Molecular markers can enable breeders to screen out plants that meet the requirements more quickly and improve the efficiency of breeding. This can shorten the cultivation cycle and improve the overall efficacy, quality and yield of Tongzi Yimucao, promoting the development and industrialization of efficient varieties.

MAS can help breeders precisely select genes related to medicinal traits in the breeding improvement of Tongzi Yimucao. MAS can also select traits that can make plants grow better and have higher yields, especially in environments with different climatic conditions or limited land resources, where it shows more advantages. MAS can also integrate multiple resistance genes into one variety, enabling the plant to resist various diseases and enhancing its survival ability and stable yield capacity under complex biological stress.

Gene editing tools such as CRISPR/Cas9 can precisely adjust specific traits at the genetic level, enhancing the efficiency and accuracy of breeding. This technology enables breeders to directly target genes and optimize them. The gradual reduction in the cost of these technologies will make them more widespread. In the future, it will be possible to cultivate varieties with high yields and strong disease resistance, as well as to carry out customized development for specific medicinal needs or special environmental conditions. The integration of this technology and method can ensure the high quality and stability of Tongzi Yimucao, and also lay a solid foundation for meeting the dual demands of the market for the efficacy and supply of traditional Chinese medicinal materials.

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### 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.

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