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Application of Superior Trait Screening and Molecular Marker-Assisted Breeding in Sapindus

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Abstract This study expounds the basic principles of screening superior traits, introduces the practices of field experiments, combines the association analysis of traits and genes, as well as whole-genome sequencing, and further clarifies the genetic mechanism behind the good traits of Sapindus. By analyzing some successful cases of Sapindus breeding, the importance of MAS technology in the breeding process is illustrated. Through MAS technology, a considerable amount of practical breeding work has been carried out, significantly enhancing the efficiency and success rate of breeding. New tools such as whole-genome selection (GS) and big data analysis may play a greater role in precision breeding in the future. This study aims to bring new ideas to the breeding of Sapindus and also provide references for the molecular breeding of other economic plants.

Keywords Sapindus; Superior trait selection; Marker-assisted selection (MAS); Gene-trait association; Whole-genome analysis

1 Introduction

Sapindus, also known as “soapberry”, is a plant with many uses and high value. Its most significant economic value lies in its ability to produce saponins. Saponins are often used in natural detergents, medicines and cosmetics because of their bactericidal and foaming effects (Gupta et al., 2017). Sapindus can also help improve soil quality, absorb carbon dioxide and prevent desertification. It is a very useful plant for sustainable land management (Jain et al., 2018). Sapindus is also often used for tree planting and afforestation because of its strong ability to adapt to the climate, especially in some areas with severe environmental degradation, and the effect is very good (Li et al., 2019).

Zhang et al. (2020) and Wang et al. (2021) hold that the key to improving plants with economic and ecological value like Sapindus lies in quickly identifying superior traits and applying advanced breeding techniques. Traditional breeding often fails to achieve satisfactory results when dealing with complex traits such as high saponin content, drought resistance, and disease resistance, which are controlled by multiple genes. However, these traits are crucial for enhancing the utilization value of Sapindus. In the research conducted by Zhang et al. in 2020, it was demonstrated that molecular marker technology has made significant progress, enabling the breeding process to become more precise and efficient. Marker-assisted selection (MAS) can combine the actually observed traits and molecular data, accelerate the breeding speed, and select good varieties more accurately (Wang et al., 2021). Jain et al. (2018) and Li et al. (2019) demonstrated that these technologies are helpful in enhancing the economic value and environmental adaptability of Sapindus, and also provide support for achieving greener and more sustainable agricultural development.

This study expounded on target traits such as high yield, strong stress resistance and high saponin content. Through genetic analysis, stable connections between these traits and molecular markers were established, and the application of MAS technology in actual breeding projects was demonstrated, hoping to improve the efficiency, accuracy and promotion possibility of Sapindus breeding. This study aims to combine the screening of superior traits with molecular marker-assisted breeding (MAS) to solve some problems encountered in traditional Sapindus breeding.

2 Foundation of Superior Trait Selection in Sapindus

2.1 Theoretical basis and current research on trait selection in Sapindus

Recent studies have found that there are significant genetic differences in the populations of *Sapindus mukorossi* in different regions. Chung and Liao (2022) completed the chromosome-level genomic assembly of Sapindus and identified many genes related to defense, growth, and development. Silva et al. (2021) also discovered the relationship between fruit traits and certain molecular markers through their research using ISSR molecular markers. These results provide an important genetic basis for trait screening.

2.2 Selection criteria for phenotypic traits and their relationship to application value

The external properties of Sapindus and its application value, especially in the production of vegetable oils and chemicals, are closely related. Liu et al. (2021) established a comprehensive evaluation system in order to select high-quality Sapindus tree species, focusing on key traits such as oil content, seed size and biomass. This system uses a method called “projection tracing” to determine how important each trait is, ensuring that the screening process is scientific and comprehensive. In 2018(b), Sun et al. also screened the germplasm resources of Sapindus based on agronomic traits and identified several superior varieties with relatively high contents of oil and saponins, which is of great value for the development of biodiesel and green chemical products.

2.3 Field trials and methods for phenotypic data collection and analysis

Xue et al. (2022) evaluated 25 traits in the Sapindus seedling population and used phenotypic variation analysis, principal component analysis (PCA), and correlation analysis to examine the extent of the differences among these traits. This method is beneficial for identifying which traits are most suitable for judging the diversity of Sapindus, thereby screening out superior individuals more quickly. Another study evaluated different Sapindus varieties using 19 agronomic traits. The results showed significant differences in yield and economic traits among them, providing useful data support for finding high-yielding and stable good varieties.

3 Role of Molecular Markers in Sapindus Breeding

3.1 Overview of molecular marker technologies and their application in plant breeding

There are many commonly used types of markers nowadays, such as ISSR and EST-SSR. These methods are widely used in the genetic research of Sapindus. The ISSR technology can be used to analyze the genetic diversity of Sapindus and identify molecular markers related to fruit traits, which is very useful for breeding efforts to improve fruit quality and oil yield. EST-SSR can be used to study population structure and help establish the core germplasm resources of Sapindus, which is very important for resource management and conservation (Fu et al., 2017). Benchimol-Reis (2023) indicates that these markers are increasingly widely used in breeding with the development of sequencing technology. New markers are constantly being developed, and marker-assisted selection (MAS) has also become easier to be applied in actual breeding.

3.2 Development and validation of markers related to target traits in Sapindus

The key to developing molecular markers related to the target traits of Sapindus is to identify those gene loci related to seed oil, saponin content in fruits, fruit quality, etc. The study by Yang et al. (2015) used ISSR labeling technology to identify 18 loci related to 13 fruit traits, including seed oil and saponin yields. They can help breeders select the desired traits more quickly and accurately. Schulman (2007) confirmed through association analysis in his early studies that there were indeed associations between these markers and the target traits, ensuring the reliability of these markers. Once these markers are successfully verified, they can be directly applied to the breeding program to enhance the superior traits of Sapindus.

3.3 Experimental evidence for the correlation between molecular markers and target traits

The study by Liu et al. (2022) used ISSR markers in *Sapindus mukorossi* and *S. delavayi* and found that some gene loci were significantly associated with traits such as seed oil and saponins. These results were also supported by EST-SSR marker analysis, which revealed the genetic differences and structures of these Sapindus populations. This information is very useful for breeding work. Verifying the relationship between these markers and traits is the key to making marker-assisted breeding (MAS) truly effective. Only when it is confirmed that these markers can accurately predict traits can they play a guiding role in breeding (Figure 1).

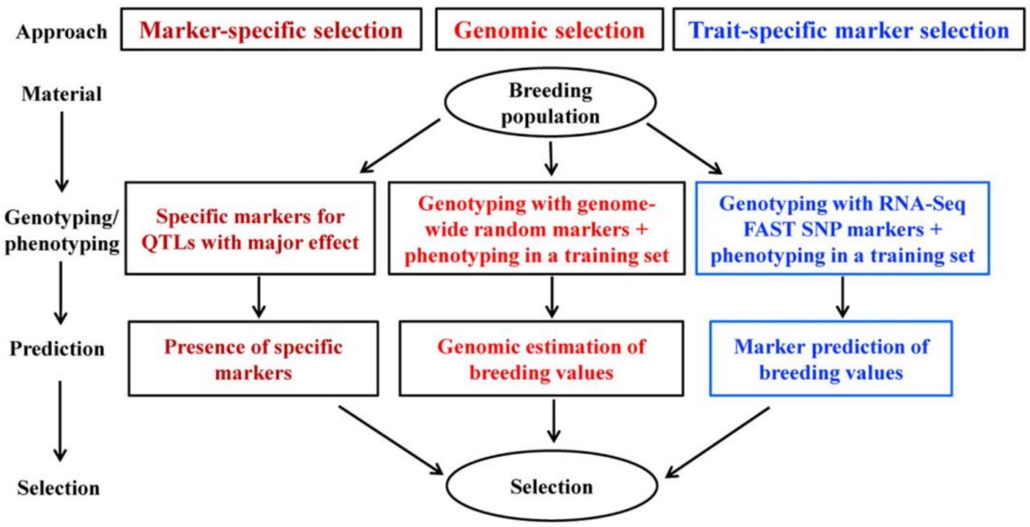

Figure 1 Genes with primary and secondary effects of the quantitative trait of interest are captured by trait-specific (FAST) SNP markers associated to rna-seq in molecular plant breeding (Adopted from Fu et al., 2017)

4 Practice of Marker-Assisted Breeding

4.1 Basic principles and workflow of marker-assisted selection (MAS)

The principle of marker-assisted selection (MAS) is to identify DNA markers related to a certain target trait, and then use these markers to track these traits in the breeding population (Bonnett et al., 2004). The advantage of this method is that without waiting for the plants to grow and then observing their performance, those plants with good genes can be selected in advance, thereby accelerating the breeding process. The general process is to first identify the markers related to traits, then verify whether these markers are reliable, and then apply them to the breeding program to select disease-resistant, high-yielding or high-quality plants (Xu and Crouch, 2008).

4.2 Practical applications of marker-assisted selection in Sapindus breeding

MAS technology can be used in the breeding of Sapindus to improve some important traits such as oil content, disease resistance and growth rate. Breeders can identify plants with target genes more quickly through DNA markers and then carry out selection and breeding. Hasan et al. (2021) found that this method has been successfully applied in other crops to enhance traits such as drought resistance and insect resistance, indicating that it also has great application potential in Sapindus breeding. However, the practical application of MAS in Sapindus has just begun at present, and more studies are needed to identify the stable traits and marker correspondence suitable for Sapindus (Liu, 2024).

4.3 Performance of MAS technology in enhancing breeding efficiency and reliability

Through this technology, breeders can select individuals with target traits in advance when plants are not fully grown, thereby accelerating the entire breeding process. The effect of MAS largely depends on whether the relationship between markers and traits is stable. However, MAS still encounters some challenges when dealing with traits controlled by multiple genes (Thavamanikumar et al., 2013). Kumawat et al. (2020) demonstrated in their study that MAS can enhance the accuracy of selection and the genetic gain of breeding, indicating its potential to help improve efficiency in the breeding of Sapindus.

5 Gene-Trait Association Analysis in Sapindus Breeding

5.1 Statistical models and tools for marker-trait association analysis

The mixed linear model (MLM) in TASSEL software is widely used in Sapindus breeding research and it works well in identifying the association between ISSR markers and fruit traits. Sun et al. 's research in 2018a identified 18 ISSR loci, which were related to 13 fruit traits, including seed oil content and saponin yield. Principal component analysis (PCA) and correlation analysis are also often used to evaluate the trait differences among different plants, which is very helpful for determining which populations are suitable for correlation analysis (Neale and Savolainen, 2004).

5.2 Key studies on target gene mapping and function prediction

There have been many new advancements in the research on the target gene localization and functional prediction of Sapindus in recent years. Jim completed the chromosome-level genome assembly of *Sapindus mukorossi* in 2024 and discovered many candidate genes related to major agronomic traits. These genomic data are helpful in identifying the key genes that affect economic traits and can also improve breeding efficiency. Breseghello and Sorrells (2006) screened out the core germplasm resources of Sapindus using EST-SSR markers in their early studies, which is very important for subsequent breeding and genetic research.

5.3 Molecular mechanisms underlying the formation of superior traits in Sapindus

The research by Ma et al. (2023) found that *Sapindus mukorossi* has obvious population structure and interspecific genetic differences. The overall genetic diversity is rich, and the populations in different regions have their own genetic characteristics. In 2019, Barreto et al. identified many key genes related to defense, growth and development through genome-wide selective sweep analysis. These genes are very important for selecting high-quality varieties. Understanding the genetic structure and geographical differentiation patterns of Sapindus is useful for formulating more scientific breeding strategies and enhancing the diversity of traits and improvement effects.

6 Case Studies on Breeding Technologies and Achievements

6.1 Breeding cases for high yield and stress resistance traits in Sapindus

Considerable progress has been made in the breeding of high-yield and stress-resistant traits of Sapindus in recent years. Researchers successfully analyzed the genetic diversity of Sapindus by using molecular marker technology and identified gene markers related to fruit traits such as seed oil and saponin yield. These markers can help quickly screen out good varieties with high-yield potential and stress resistance, and improve the efficiency of breeding (Germanà, 2011).

6.2 Significant trait improvements achieved through MAS technology

Marker-assisted selection (MAS) plays a crucial role in improving important traits in the breeding of Sapindus. MAS can precisely select plants with excellent characteristics by finding the relationship between molecular markers and target traits. This technology is highly suitable for accelerating the breeding speed and can effectively enhance the fruit quality and oil content (Tester and Langridge, 2010). The application of MAS has helped breed some excellent Sapindus varieties, which have better agronomic traits and significantly increased commercial value (Figure 2) (Godwin et al., 2019).

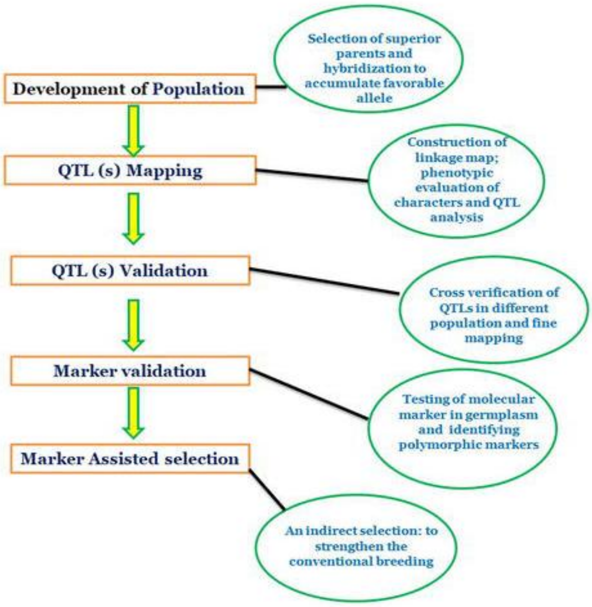

Figure 2 Flow chart of MAS technical marking development(Adopted from Kumawat et al., 2020)

6.3 Challenges and solutions in practical breeding applications

One of the biggest problems faced by MAS technology in Sapindus breeding is how to truly apply the research results to breeding, which will encounter limitations in terms of logistics and genetics in the process. Su et al. (2019) found that complex traits such as stress resistance are influenced by the environment and the interactions among multiple genes. Therefore, it is necessary to have a deeper understanding of the relationship between genotypes and the environment, as well as the interactions among genes (also known as abogenicity). To solve these problems, it becomes very important to develop high-throughput phenotypic and genotypic analysis techniques. There are also some more efficient breeding strategies, such as backcrossing breeding and enrichment of target traits in the F2 generation population, which have also been proven to improve breeding efficiency (Thomson et al., 2009).

7 Potential of Whole-Genome and Big Data Analysis in Sapindus Breeding

7.1 Progress and applications of whole-genome sequencing in Sapindus

Whole genome sequencing (WGS) can provide detailed genetic information and help breeders formulate breeding strategies more precisely. WGS can be used in the breeding of Sapindus to identify genetic markers related to target traits and improve breeding efficiency. Meuwissen et al. (2021) hold that by using these data, the gene regions (QTL) related to traits can be located more accurately, which is very beneficial for understanding the genetic basis of the complex traits of Sapindus. Combining WGS with some genomic prediction models (such as GBLUP and Bayesian models) can also improve the accuracy of trait prediction, especially among different Sapindus species.

7.2 Role of big data and artificial intelligence in phenotype-genotype analysis

In the breeding of Sapindus, a large amount of genotype and phenotype data has been accumulated. These data can help predict the expression of traits more accurately and also make the selection process more efficient. Especially machine learning (ML) algorithms are very useful when processing and analyzing these huge amounts of data. They can identify the genetic structure behind traits and provide strong support for breeding decisions (Tempelman, 2015). Singh and Prasad demonstrated in their 2021 study that the addition of AI is helpful for discovering new genetic associations and predicting the results of complex traits with higher precision, thereby enhancing the efficiency of Saponus breeding.

7.3 Future prospects of genomic selection (GS) in precise Sapindus breeding

GS can select complex traits more accurately by using whole-genome data, accelerate the breeding process and improve the breeding effect. The combination of GS and WGS (whole genome sequencing) data can identify key genetic variation points and directly apply these variations in breeding to achieve the target traits (De Los Campos et al., 2013). Ros-Freixedes et al. (2022) indicated that sequencing technology is becoming increasingly advanced and the cost is getting lower and lower. This means that the application of GS in soapy seed breeding will increase more and more, and it is expected to accelerate the development of excellent new varieties such as disease resistance and high yield.

8 Concluding Remarks

This study demonstrates how to apply superior trait screening and molecular marker-assisted breeding methods to Sapindus plants. There is a study found significant genetic differences between *Sapindus mukorossi* and *S. delavayi* through the use of ISSR markers. These plants have high economic value because they are rich in compounds such as vegetable oil and saponins. The study also established the association between ISSR markers and some important fruit traits, laying the foundation for breeding efforts to improve these traits. The application of these markers provides a reliable tool for screening high-quality resources and significantly improves the efficiency of Sapindus breeding.

Future research should pay more attention to expanding the genetic basis of Sapindus. Introducing more germplasm resources from different sources can enhance the stability and adaptability of the breeding program. Combining high-throughput phenotypic determination techniques with MAS (marker-assisted selection) can enhance the accuracy and efficiency of breeding. In actual breeding, it is necessary to develop a cheaper and more practical genotyping system to make the MAS technology easier to be promoted and applied, so that both breeders and farmers can benefit better from this technology.

Marker-assisted selection (MAS) has great potential in accelerating the breeding speed and improving the accuracy of target trait selection. Nowadays, DNA labeling technology and QTL localization technology are becoming increasingly advanced, providing breeders with powerful tools that can more effectively improve complex traits and enhance variety performance. As these technologies continue to develop, they are also expected to play an important role in addressing global food security issues and be used to cultivate new crop varieties that are high-yielding, stress-resistant and more nutritious, promoting agricultural progress. Combining molecular breeding with traditional methods can also make agriculture more efficient and sustainable.

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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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