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Genetic Diversity Analysis and Superior Gene Mining of *Sapindus mukorossi* Germplasm Resources

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Abstract This study conducted a relatively comprehensive analysis of the germplasm resources of *Sapindus mukorossi*, examined its genetic diversity, explored the structure among different populations and their evolutionary relationships, identified some functional genes related to saponin content and stress resistance, and evaluated the performance of superior germplasm in different ecological environments. This study also confirmed through the association analysis of genotypes and phenotypes which genes might affect saponin content and stress resistance. This study reveals the diversity of *Sapindus mukorossi* germplasm and the genetic basis behind these good traits, aiming to provide a theoretical basis for future molecular breeding.

Keywords *Sapindus mukorossi*; Genetic diversity; Superior genes; Saponin content; Molecular markers

1 Introduction

Sapindus mukorossi (also known as the soap nut tree) is an economic tree species with many uses. The fact that its fruit contains natural saponins makes it very popular in industries such as laundry, medicine and cosmetics (Upadhyay and Singh, 2012; Li et al., 2013; Wei et al., 2020; Sochacki and Vogt, 2022). Zhao et al. (2019) believed that if the germplasm resources of *Sapindus mukorossi* have rich genetic diversity, it is helpful for its protection and laying a good foundation for subsequent development and utilization. Understanding these genetic differences is useful for breeders to select varieties with high saponin content and strong stress resistance and to expand its application in industry (Chang et al., 2021).

Gao et al. (2023) proposed that the fruit saponin content of *Sapindus mukorossi* is high, and its economic value is highly valued. The improvement of economic traits is inseparable from the rich genetic diversity in germplasm resources. Mahar et al. (2011b) and Xue et al. 's research in 2022 found that *Sapindus mukorossi* populations in different regions showed significant differences, with the highest genetic diversity in the southwest region. This is beneficial for the adaptation and survival of the species itself and providing more options for subsequent breeding (Sun et al., 2018a; Liu et al., 2022).

Understanding the genetic diversity of *Sapindus mukorossi* can provide a scientific basis for breeding high-quality varieties and is a key step in improving economic benefits. Sun et al. (2018b) and Liu et al. (2021) demonstrated that by identifying genes related to high saponin content or stress resistance, molecular breeding methods can be utilized to cultivate stronger and more productive *Sapindus mukorossi*. This genetic information is very useful for the screening of high-quality germplasm and the breeding of good varieties suitable for various environments (Mahar et al., 2011a; Xue et al., 2022).

This study will analyze the genetic diversity of *Sapindus mukorossi*, identify the dominant genes suitable for breeding, understand its genetic structure and variation, and also provide a reference for formulating more scientific protection and utilization strategies. This study will also explore the practical application of molecular markers in germplasm screening, laying the foundation for the future promotion of molecular-assisted breeding techniques in *Sapindus mukorossi*.

2 Distribution and Ecological Characteristics of *Sapindus mukorossi* Germplasm Resources

2.1 Distribution of *Sapindus mukorossi* germplasm resources

Sapindus mukorossi is mainly distributed in the subtropical and tropical regions of Asia. It is relatively adaptable to warm climates and diverse ecological environments and can grow widely in these areas (Sun et al., 2017; Liu et al., 2021b; Liu et al., 2021c). *S. mukorossi* is the most widely distributed species in the *Sapindus* genus, concentrated in the central and eastern regions of China. *S. delavayi* is mainly distributed in southwestern China, while *S. rarak* is mainly found in Southeast Asia (Figure 1). Liu et al. (2021d) and Wang et al. (2020) hold that the ability of *Sapindus mukorossi* to adapt to different environmental changes is also one of the reasons why it can survive in multiple regions.

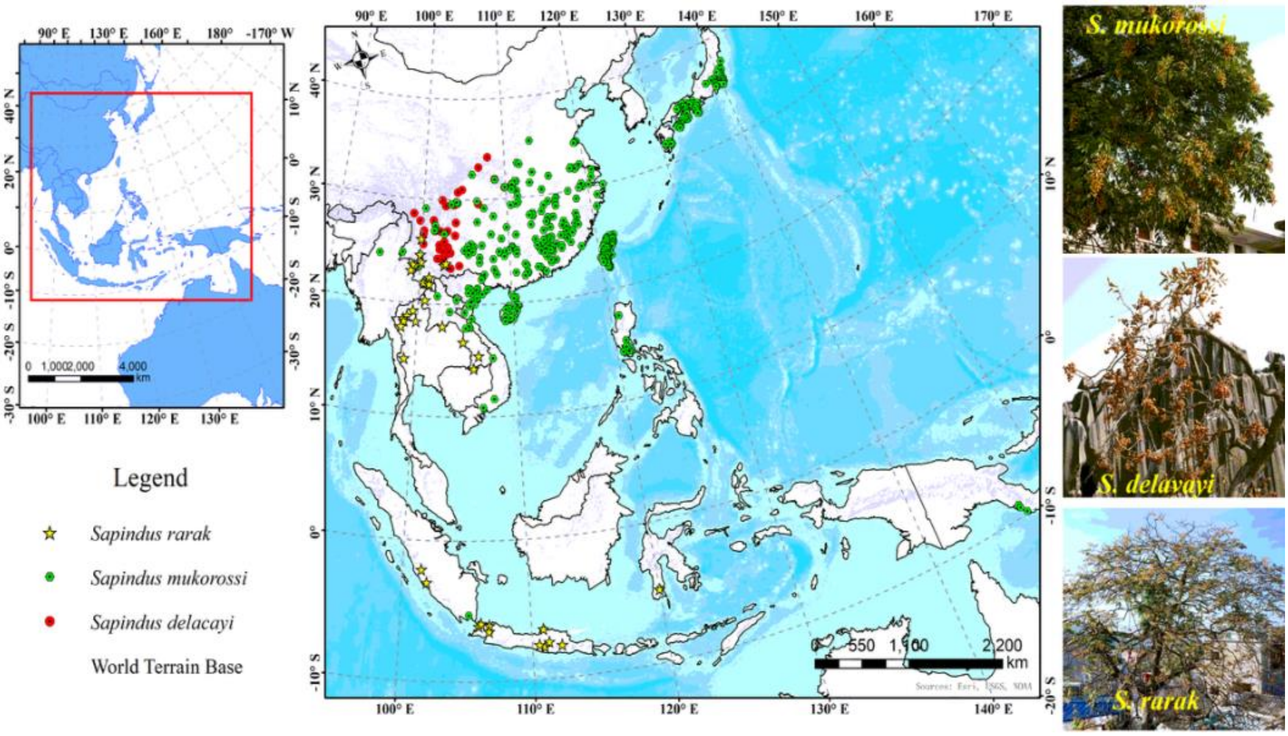

Figure 1 Spatial distribution of occurrence records of *S. mukorossi*, *S. delavayi*, and *S. rarak* (Adopted from Liu et al., 2021d)

The main production areas of *Sapindus mukorossi* in China include provinces such as Fujian, Zhejiang and Guizhou. These places have a warm climate and belong to the climate type between the temperate and tropical zones. The precipitation is generally medium to high, and the soil is relatively fertile. The pH value is mostly between 5.6 and 7.6. This soil condition is very suitable for the growth of *Sapindus mukorossi* (Liu et al., 2021b; Liu et al., 2021c). Liu et al. (2021c) also found that the soil moisture content in these areas was generally between 40 and 140 millimeters, providing a good water basis for the normal growth and yield of *Sapindus mukorossi*.

2.2 Phenotypic diversity of *Sapindus mukorossi* germplasm

The germplasm resources of *Sapindus mukorossi* vary greatly in appearance and traits. Different varieties have obvious differences in tree height, leaf size and fruit weight (Sun et al., 2017; Gao et al., 2018; Wang et al., 2020). The size and saponin content of fruits vary greatly, which is very important for breeding work because better varieties need to be selected for promotion and cultivation during breeding (Liu et al., 2021b; Liu et al., 2022; Song et al., 2023).

The research by Liu et al. (2021b) showed that the saponin content in the fruit of *Sapindus mukorossi* varied between 4.14% and 27.04%, and the oil content in the seeds ranged from 26.15% to 44.69%. Sun et al. (2017) and Liu et al. (2021b) hold that this difference is mainly influenced by both genes and the external environment. It is crucial to accurately assess these traits before breeding *Sapindus mukorossi* varieties with high yield and good quality.

2.3 Impact of ecological factors on genetic diversity

Climatic conditions such as temperature and precipitation can affect the growth performance and adaptability of plants, leading to genetic differentiation in *Sapindus mukorossi* populations in different regions (Diao et al., 2014; Liu et al., 2021d; Wang et al., 2022). Liu et al. (2021b) and Liu et al. (2021c) hold that the pH value and moisture content of the soil can affect the growth status of *Sapindus mukorossi*, and these differences are also directly related to the genetic variations of populations in different regions. Sun et al. (2018) and Liu et al. (2022) found that geographical isolation is equally important and it may cause long-term differentiation among populations in different regions.

3 Methods and Techniques for Genetic Diversity Analysis

3.1 Research materials and sampling strategy

The studies of Mahar et al. (2011), Ba (2014), and Liu et al. (2022) all found that *Sapindus* plants are distributed in many places (such as China, India, etc.), and there are significant genetic differences among the populations in these regions. Extensive sampling is beneficial for breeders and conservationists to better grasp germplasm resources and lay the foundation for subsequent selection and breeding as well as resource conservation.

Liu et al. (2021a) demonstrated that the phenotypic trait data of *Sapindus mukorossi* were collected through agricultural morphology methods, and the assessment contents included the size of the fruit, the oil content in the seeds, and the saponin level in the fruit, etc. These traits will be recorded first, and then the outstanding germplasm resources will be identified through correlation analysis and principal component analysis. Sun et al. (2018a) found that the research would also take into account the phenotypic plasticity and adaptability of *Sapindus mukorossi*, as these factors would also affect its economic value.

3.2 Application of molecular marker technologies

SSR and SNP markers can detect very subtle genetic differences when analyzing the genetic diversity of *Sapindus mukorossi*. SSR markers are often used to evaluate the genetic diversity and population structure of *Sapindus mukorossi*. Studies have found that they can reveal obvious intraspecific and interspecific variations. Sun et al. (2018b) and Liu et al. (2022) hold that the advantages of such markers lie in their codominant nature, large amount of information, high polymorphism, and suitability for studying kinship and genetic diversity.

High-throughput sequencing technology can be used to obtain more comprehensive genetic information. It is useful for in-depth understanding of the changes in the entire genome, identifying those candidate genes related to agronomic traits, and revealing the evolutionary history of the *Sapindus mukorossi*. Xue et al. (2022) demonstrated that the introduction of high-throughput sequencing can provide a more detailed analysis of genetic differences among germplasm resources and more accurately select superior materials suitable for breeding.

3.3 Methods for evaluating genetic diversity

Statistical indicators such as the Shannon index and the Nei index are beneficial for understanding the genetic differences within and between populations. The Shannon information index is often used to measure the genetic diversity of different populations. The research of Mahar et al. (2011b) shows that *Sapindus mukorossi* in different geographical regions has significant changes in polymorphism and genetic differences. Sun et al. (2018a) found that the Nei genetic diversity index is often used to quantitatively assess the degree of variation and has been used to analyze the diversity parameters of *Sapindus* plants in some studies based on ISSR markers.

Methods such as UPGMA cluster analysis and Dice genetic similarity coefficient are often used to analyze the genetic similarity and distance between germplasms. Mahar et al. (2011b) and Ba (2014) found that UPGMA clustering can divide different populations into several groups based on genetic distance, and the results are often related to geographical distribution. That is to say, populations from similar areas are more likely to be classified into one category. These analytical methods are very useful for understanding the genetic relationships and differences among different germplasm resources.

3.4 Population structure and evolutionary analysis

Sun et al. (2018b) found that PCA can represent complex genetic data in the form of images, allowing the genetic differences between different populations to be visually observed, and also analyze whether these differences are related to ecological and environmental factors. Liu et al. (2022) indicated that the Fst value is used to measure the degree of genetic differentiation among different populations, and the higher the value, the greater the difference between populations.

ISSR markers are widely used in the genetic analysis of *Sapindus* plants. They can effectively distinguish the genetic structure among different species and populations and reveal obvious genotype differences and evolutionary directions (Sun et al., 2018a; Sun et al., 2018b). Xue et al. (2022) demonstrated that these research results are helpful for understanding the evolutionary process and environmental adaptability of *Sapindus mukorossi*, as well as enabling breeders to identify superior germplasms with greater utilization value.

4 Mining and Functional Analysis of Superior Genes in *Sapindus mukorossi*

4.1 Functional genes related to saponin content

The chromosome-level genome of *Sapindus mukorossi* has been assembled, providing a complete reference framework for identifying genes related to saponin synthesis. In the 2022 study by Xue et al., genomic data revealed that the *Sapindus mukorossi* contains many genes related to plant defense, growth and development, and these genes are very likely to include the key parts that control saponin synthesis.

Liu et al. (2021a) and Xue et al. (2022) conducted gene expression analysis on germplasm with high saponin content using RNA-Seq technology and identified the core genes that might regulate saponin synthesis. This method is beneficial for a better understanding of the genetic basis of saponin content and also provides technical support for screening high-quality germplasm with higher saponin yield.

4.2 Genes related to stress resistance

Identifying genes related to drought resistance, salt tolerance, heat tolerance and other stress resistance is crucial for breeding *Sapindus mukorossi* varieties with strong adaptability. Xue et al. (2022) have identified a number of candidate genes that may be involved in plant defense responses through genome-wide analysis. These genes may be crucial in enhancing plants' resistance to adverse environments.

Studies have screened and verified the expression of candidate genes in *Sapindus mukorossi* germplasm with strong stress resistance, and found that these genes do have potential in enhancing the tolerance of plants to external environmental stress, highlighting the importance of genetic diversity and also indicating the key role of certain specific genes in adapting to different adverse conditions (Mahar et al., 2011; Ba, 2014; Sun et al., 2018b).

4.3 Validation technologies for functional genes

RT-qPCR can quantify the expression of a certain target gene and verify whether it is really related to important economic traits such as saponin content or stress resistance. Sun et al. (2018a) and Xue et al. (2022) argued that these target genes are often preliminarily screened out in genome-wide analysis or selective dissection studies, and RT-qPCR is used to confirm whether these genes do play a role in key traits.

Mahar et al. (2011a) and Xue et al. (2022) found that gene editing technology can precisely modify specific loci in the genome and knockout a gene involved in saponin synthesis or stress resistance pathways, thereby directly verifying its role in plant traits. The combined use of gene editing technology and traditional breeding methods can accelerate the breeding process of high-yield and stress-resistant *Sapindus mukorossi* varieties and enhance their commercial utilization and environmental adaptability.

4.4 Candidate gene association analysis

Candidate gene association analysis is to obtain genotype data through molecular markers, and then conduct association analysis on these data with phenotypic traits such as saponin content and stress resistance in fruits. Sun et al. (2018a) and Liu et al. (2021a) demonstrated that in this way, genetic loci related to key economic traits can be identified, which is beneficial for locating the genes that play a role and providing a genetic basis for breeding work.

Combining association analysis and genetic diversity assessment can help understand the extent to which certain genes influence the target traits. The research by Mahar et al. (2011b) and Sun et al. (2018a) utilized ISSR markers to identify loci related to saponin synthesis and other economic traits, revealing the genetic mechanisms behind these traits. By understanding these genetic factors, breeders can formulate more targeted strategies, focusing on enhancing key indicators such as saponin content and stress resistance of *Sapindus mukorossi*, and promoting its application value in biodiesel, pharmaceutical development and biochemical engineering fields.

5 Screening and Evaluation of Superior Germplasm in *Sapindus mukorossi*

5.1 Comprehensive trait evaluation indicators

Xue et al. (2022) found that *Sapindus mukorossi* exhibits distinct biological characteristics from flowering to fruiting. Detailed records of its morphology and growth habits provide a solid foundation for subsequent breeding, cultivation, and medicinal development (Figure 2). Sun et al. (2018a) and Liu et al. (2022) indicated that germplasms with high saponin content have attracted attention due to their applications in biodiesel and the pharmaceutical fields, and the high content of oil in seeds is related to the production efficiency of biodiesel. Mahar et al. (2011b) and Sun et al. (2018b) hold that the stress resistance ability that can grow in various environments is an important factor in evaluating the quality of germplasm, and it is related to the sustainable planting performance in different ecological areas.

Figure 2 Representative photographs of *S. mukorossi* (Adopted from Xue et al., 2022)

Image caption: a Mature *S. mukorossi* tree in its natural habitat. b Flowers. c, d Fruits. Bar =1 cm (Adopted from Xue et al., 2022)

5.2 Screening results of germplasm resources

Molecular marker techniques such as ISSR and EST-SSR are often used to identify genetic variations related to these key traits (Sun et al., 2018a). Liu et al. (2021a) screened out a variety of outstanding germplasms through these techniques. The germplasms numbered 80, 110 and 112 in the study performed well in terms of yield and saponin levels, and were ideal materials for the breeding program. Mahar et al. (2011b) pointed out that the superior performance of some germplasms in specific regions indicates that regional adaptability needs to be considered when selecting superior germplasms. Liu et al. (2021a) hold that the goal of germplasm screening is to make full use of the existing trait differences and improve the overall quality and yield of *Sapindus mukorossi* through breeding. In their early research in Himachal Pradesh, India, Bahar and Singh (2007) identified several sources of high-quality seeds through indicators such as seed weight, germination rate and viability index.

5.3 Regional adaptability evaluation

The research of Sun et al. (2018b) found that different germplasm of *Sapindus mukorossi* exhibited differently in regions with different precipitation and temperature conditions. The germplasm of subgroup 1 in the study is more suitable for areas with moderate precipitation and lower temperatures, and the germplasm of subgroup 2 can better cope with the environment with large rainfall fluctuations. Areas with moderate climate and moderate rainfall are more suitable for promoting the cultivation of high-yield and high-quality *Sapindus mukorossi* varieties (Mahar et al., 2011a; Sun et al., 2018b). Bisht et al. (2016) hold that by combining the determined genetic markers and environmental adaptability, new varieties with high saponin content and excellent comprehensive traits can be developed in a targeted manner.

6 Development and Utilization of Superior Germplasm Resources of *Sapindus mukorossi*

6.1 Breeding techniques for superior germplasm

Through the observation and selection of different seed sources, germplasm resources with high saponin content and strong stress resistance can be screened out (Xue et al., 2022; Liu et al., 2022). Asexual reproduction is suitable for preserving and replicating these excellent traits. It can maintain the stability of germplasm without changing genes and is suitable for maintaining the genetic consistency of high-quality plants (Mahar et al., 2011b; Singh et al., 2015; Singh et al., 2019).

The research by Sun et al. (2018b) and Liu et al. (2021a) pointed out that different genotypes behave differently under different climatic conditions. Selecting the appropriate genotype is crucial for achieving large-scale and efficient planting. Mahar et al. (2011a) and Sun et al. (2018a) demonstrated that the application of molecular markers is helpful for breeders to screen out high-yield and high-quality varieties more quickly in this process, and also provides technical support for establishing a large-scale production system.

6.2 Industrial applications of superior germplasm

Sapindus mukorossi has attracted extensive attention in the detergent industry due to its high saponin content in its fruit, and it is a natural and environmentally friendly alternative resource. Liu et al. (2021a) and Liu et al. (2022) have identified several high-quality germplasms with high saponin yields, which are very suitable for the industrial development of washing products. Mahar et al. (2011b) hold that these natural saponin products are greener and safer compared with traditional synthetic detergents, meeting the market demand for sustainable natural products.

Some genotypes can still maintain a good growth state in an environment with high altitude and large variations in rainfall, and are particularly suitable for greening projects in ecologically fragile areas (Ba, 2014; Sun et al., 2018b). The application of these germplasms is beneficial to enhancing plant diversity and also plays an active role in the restoration of degraded land and landscape stability (Mahar et al., 2011a; Xue et al., 2022).

6.3 Recommendations for germplasm resource conservation and development

Sun et al. (2018a) and Liu et al. (2022) both hold that germplasm resource banks and gene banks can centrally preserve high-quality germplasm and key genes discovered in various studies, playing a role in long-term storage and resource management. Establishing such a preservation system can ensure that genetic materials with excellent traits such as high saponin content and strong stress resistance are not lost, providing stable genetic resource support for future breeding improvement, basic research and industrial promotion (Mahar et al., 2011b; Ba, 2014).

Sun et al. (2018a) and Liu et al. (2022) demonstrated that superior genes related to key traits such as saponin yield and stress resistance have been able to be identified more efficiently with the development of molecular breeding techniques. The promotion of technology has enhanced the breeding efficiency and provided the possibility for the rapid selection and breeding of new *Sapindus mukorossi* varieties. The new variety has stronger application potential in fields such as detergents, biomedicine and ecological restoration, and has higher industrial and ecological value. Liu et al. (2021a) and Xue et al. (2022) hold that continuously strengthening related research and investment in molecular breeding is beneficial for ensuring the sustainable utilization of *Sapindus mukorossi* germplasm resources and promoting their development in multiple fields.

7 Challenges and Future Directions

7.1 Technical bottlenecks in genetic diversity research

Xue et al. (2022) held that the genomic structure of *Sapindus mukorossi* is relatively complex, with chromosome-level genomic assembly covering approximately 391 Mb, requiring powerful computing tools and efficient data analysis processes to handle the huge amount of information. Although many candidate genes related to important agronomic traits such as saponin content and stress resistance have been found, whether these genes truly play a role still needs to be verified. The functional verification process is rather complex, with a long experimental period and high technical requirements (Sun et al., 2018a).

7.2 Promotion challenges in utilizing superior genes

Although many genetic markers have been developed and core germplasm banks have been gradually established, the truly high-yield and stable varieties suitable for commercial promotion are still limited (Liu et al., 2021a). How to effectively utilize the genetic differences and trait variations in local populations in actual breeding still requires more systematic assessment and technical means to promote the selection and promotion of high-quality germplasm (Mahar et al., 2011a; Mahar et al., 2011b).

7.3 Future research directions

The combined application of genomics and transcriptomics requires more attention and in-depth exploration of functional genes related to key economic traits (Sun et al., 2018a; Sun et al., 2018b; Fang, 2024). The development of an intelligent breeding platform and the utilization of advanced tools such as artificial intelligence and machine learning can enable rapid screening of germplasm and precise prediction of the optimal breeding combination, thereby enhancing the efficiency and accuracy of the entire breeding process (Liu et al., 2021a; Liu et al., 2022; Wu, 2024).

8 Concluding Remarks

Studies on the genetic diversity of *Sapindus mukorossi* have revealed its rich population structure and significant genetic variations. *S. mukorossi* has a relatively high genetic diversity within the species and a clear population structure. Compared with *S. delavayi* and *S. rarak*, it shows more abundant genetic variation characteristics and has the potential to be used as a breeding material. Researchers have successfully identified multiple candidate genes related to key agronomic traits through chromosome-level genome assembly. Studies based on ISSR have also identified genetic loci related to economic traits such as fruit quality and yield.

The genetic resources of *Sapindus mukorossi* are crucial for its long-term sustainable utilization and industrial development. Establishing a core germplasm bank can effectively preserve most of the genetic variations of species and achieve efficient management and protection of resources. The rich genetic diversity within the S. mukorossi population provides a sufficient genetic basis for breeding and is useful for selecting and breeding new varieties with high yield and high quality. Diversification strategies such as the construction of germplasm resource banks and in-situ conservation are all beneficial for preserving the key genetic resources needed for future innovation and breeding.

The introduction of modern molecular breeding techniques has provided new possibilities for the green transformation and sustainable development of the *Sapindus mukorossi* industry. The integration of molecular markers and genetic information can increase the added value of *Sapindus mukorossi* products in fields such as biodiesel, natural detergents and medicine, and breed more environmentally adaptable varieties. These measures are expected to enhance the industrial competitiveness of *Sapindus mukorossi* and promote its development under the dual goals of ecology and economy.

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Conflict of Interest Disclosure

The author affirms 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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