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The Optimization of Fast Propagation Technology for *Sapindus* Seedlings and Its Resistance Enhancement Study

Huixian Li, Jie Zhang
Institute of Life Sciences, Jiyang Colloge of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China
Corresponding email: jie.zhang@jicat.org

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Abstract This study explores good methods to accelerate the reproduction of soapberry seedlings, if they can be made more resistant to various adverse environments. In order to make the seedlings survive easily, grow roots and maintain their original excellent characteristics, efficient propagation methods that can be used both in the laboratory and outdoors were studied. How to treat the seeds before sowing was investigated, which can make the seeds germinate better and the seedlings grow stronger. This is very important for artificial planting forests. This study also analyzed the ecological and economic value of the acacia tree, as well as the difficulties encountered during the cultivation process, and its role in protecting biodiversity and promoting sustainable agriculture. This research aims to enable better cultivation and utilization of acacia trees, which not only helps protect the ecological environment but also plays a role in industry.

Keywords *Sapindus*; Fast propagation; Genetic integrity; Abiotic stress; Biotic stress

1 Introduction

Seedless trees, often called “soap seeds” by people, are a type of plant that includes both tall trees and short shrubs. They are of great value both in medicine and for making money. The two plants, the three-angled cypress and the wood-angled cypress, are frequently used in traditional medicine passed down from our ancestors and also have great potential in pharmaceutical factories. People value these plants because the fruits they bear contain a lot of saponins, which can be used to make natural cleansers and various things beneficial to the body (Singh et al., 2010; Asthana et al., 2011). Nowadays, many factories need these plants. Therefore, it has become particularly important to work out good propagation methods to ensure that the plant resources are always sufficient and to protect them well.

The method of rapidly propagating plants is crucial for growing crops and trees, so as to meet the increasing demand for plants and grow large areas of forests more quickly. Methods such as rapid propagation and cutting propagation can grow many plants that meet the requirements at once, and also ensure that their varieties are pure and they all look similar (Yang, 2010; Asthana et al., 2011). For instance, the method of indoor reproduction enables plants to grow rapidly in a suitable environment, which is particularly important for disease-treating plants like acacia (Singh et al., 2010). Moreover, adjusting the reproductive conditions properly can make plants grow roots more easily and live better, which is crucial for successfully planting forests (Yang, 2010; Haider et al., 2016).

This study will explore methods to make the young seedlings of the soapberry grow faster and be more resistant to diseases. This requires researching good methods for reproduction both indoors and outdoors, making it easy for seedlings to survive, grow roots, and retain their original good genes. This transaction study will also analyze how to handle seeds before sowing to make them germinate more easily and the seedlings grow stronger, so that the forest can be better planted. This research aims to ensure that seedless trees can be well cultivated and utilized all the time, which can not only protect them well but also provide raw materials for factories.

2 Species of Acacia Plants and Their Ecological and Economic Functions

2.1 Introduction to plant species of the genus *Acacia*

Seedless trees, also known as soaps, are a type of deciduous tree of varying heights and belong to the seedless family. This type of plant has its own characteristics, such as having special leaves and flowers, and also bearing round and plump fruits. These fruits contain saponins that can act as natural cleaners (Gao et al., 2023). There are several members in this family. They look quite different and can adapt well in different environments (Liu et al., 2021c; Gao et al., 2023). Take the seedless tree for example. It is very common in the south of our country. Because there are many saponins in its fruit, it is particularly valuable (Figure 1) (Sun et al., 2018; Zhao et al., 2019).

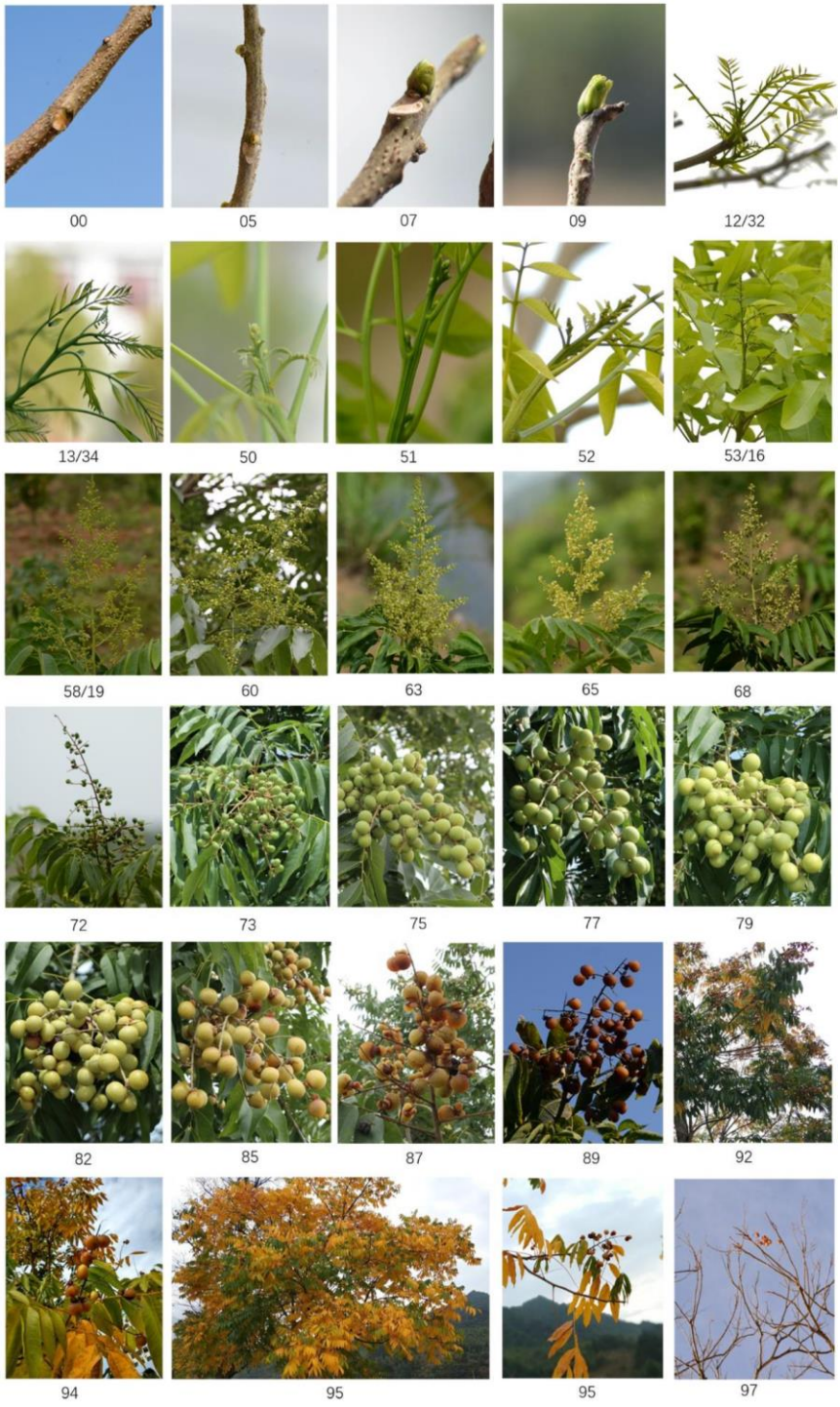

Figure 1 Phenological growth stages of *S. mukorossi* according to the BBCH scale (Adopted from Zhao et al., 2019)

Different seedless trees prefer different growing environments and are distributed in different places. Some can grow at high altitudes, while others require specific light conditions to grow well (Sun et al., 2018; Liu et al., 2021c). These different adaptability abilities indicate that the plants of this family are diverse, which also enables them to survive and function in different places.

2.2 Ecological and economic significance

Plants of the genus *Acacia* are particularly important both ecologically and economically. From an ecological perspective, they can grow well in many environments, and the saponins in their leaves can also drive away pests. Therefore, people will use them to replant trees for greening and restore the land (Liu et al., 2022; Souza et al., 2023). Economically, soap pods are of great use. They can be used to make traditional medicine, soap, and as raw materials for the production of biodiesel and other chemical products (Liu et al., 2021a; Liu et al., 2021b; Liu et al., 2022). Especially the fruits of seedless trees are often used to extract saponins. Saponins are not only an important component of natural cleaners, but also can be used in pharmaceutical factories (Zhao et al., 2019; Liu et al., 2021b).

In addition to making money directly, seedless trees are also helpful for environmental protection. They can make the soil more solid and provide a place for many small insects to live, which is very beneficial for protecting biodiversity (Souza et al., 2023). Because of its many uses, the seedless tree is crucial in both local economic development and ecological protection efforts.

2.3 Challenges faced in cultivation and propagation

Although seedless trees have so many benefits, it is not easy to plant them and have them reproduce. A major problem is that because there are no varieties that are particularly capable of bearing fruit and have stable yields, the harvest varies each year, affecting the income of seedless trees (Liu et al., 2021a). Moreover, with the current climate change, their growth environment has also been damaged, resulting in a decreasing number of seedless tree varieties. Therefore, it is necessary to find ways to protect the superior variety resources of these plants (Liu et al., 2021b).

Another problem is that different subtrees have difficulty adapting to the changing environment. For instance, due to climate change, some places may no longer be suitable for growing seedless trees in the future (Liu et al., 2021b; Liu et al., 2021c). Come up with new management methods and select varieties that can withstand environmental changes. Nowadays, people are striving to find and cultivate better varieties to solve these problems and make seedless trees grow better and have higher yields (Liu et al., 2021a; Liu et al., 2022)

3 Research Status of Propagation Methods for Soapberry Seedlings

3.1 Traditional communication technologies

In the past, the common methods for growing soapberry seedlings included sowing, cutting and grafting. Sowing is the most common. To make the seeds germinate better, people will do some treatment on the seeds before sowing. For example, soaking the seeds in hot water for a short while can make the seeds germinate more easily in the nursery and the seedlings can grow better (Haider et al., 2016). Also, soaking seeds in cold water or treating them with chemical agents can also make the seeds germinate earlier and grow stronger (Mundhe and Shamet, 2011; Swaminathan and Revathy, 2013).

Cutting propagation is to cultivate new plants using the branches or stems of plants. By adjusting different cultivation conditions and treatment methods, people have increased the success rate of root formation by cuttings. For instance, after treating the 15-centimeter-long branch tips with rooting powder, almost over 92% of them could grow roots (Yang, 2010). The advantage of this method is that the success rate is relatively high, and the newly grown plants look the same as the original ones.

3.2 Limitations of traditional methods

Although these old methods are used a lot, they also have quite a few disadvantages. Due to the varying quality of seeds and environmental changes, seedlings grown by sowing grow slowly and are prone to death. Even if the

seeds were treated in advance, the germination and subsequent growth conditions were unstable, which caused trouble for large-scale planting (Swaminathan and Revathy, 2013; Haider et al., 2016). Moreover, it takes a long time for the seedlings to be transplanted from being planted, which is not very suitable for large-scale commercial cultivation.

Although cuttings and grafting are sometimes quite effective, they also have their problems. They have relatively high requirements for their growing environment. If not well cared for, it will be very difficult for new seedlings to survive. Whether these methods can succeed largely depends on whether the growers have good skills and the quality of the plant materials used for propagation. Therefore, they are not very suitable for large-scale planting (Yang, 2010; Mundhe and Shamet, 2011).

3.3 Progress in communication methods

Recently, people have developed some new breeding techniques, such as artificial breeding, rapid breeding and cloning breeding, precisely to address the shortcomings of the old methods. For instance, artificial cultivation can quickly grow soapberry seedlings in a well-controlled environment, ensuring that the new seedlings grow exactly the same as the original ones and have a high survival rate. Research has found that cultivating a part of the plant in an artificial cultivation environment can make the new buds grow fast and in large quantities, and the final transplanting can also survive (Figure 2) (Asthana et al., 2011; Srinivas et al., 2014).

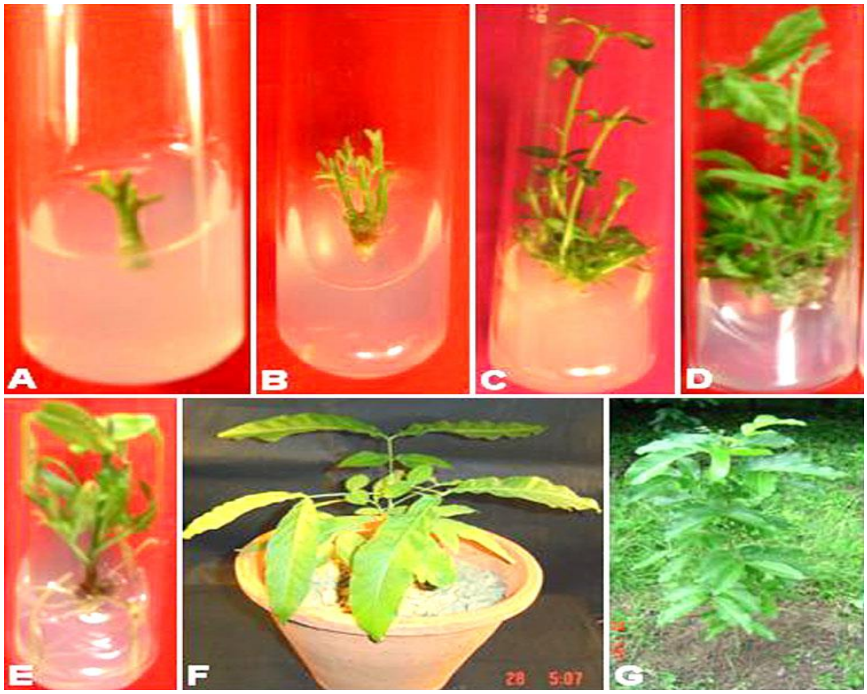

Figure 2 Micropropagation of *Sapindus trifoliatus* L. using seedling node explants (Adopted from Asthana et al., 2011)
Image caption: a Seedling node showing bud breaking on MS medium containing 1.0 mg l-1 BAP after 12 days of culture. b Multiple shoot formation from seedling node on MS medium containing 1.0 mg l-1 BAP after four weeks of incubation.c Multiple shoot induction on mother explant after three successive transfers on MS medium containing 1.0 mg l-1 BAP. d Multiple shoot formation from nodal segment excised from in vitro raised shoots on MS medium containing 1.0 mg l-1 BAP after third subculture. e Rooting of microshoot which was cultured on MS medium containing 1.0 mg l-1 IBA for 24 h and then transferred to the same medium without PGR. f Plantlet acclimatized and established in earthen pot containing garden soil. g Plant growing in field condition after 6 months of transplantation (Adopted from Asthana et al., 2011)

Rapid cultivation is a type of artificial cultivation that can produce many plants that look the same and are free from diseases and pests at once. This method uses special agents to help the stems of plants grow and take root, so that the survival rate is very high when transplanting (Singh et al., 2010). Cloning and breeding is to make the newly grown plants exactly the same as the original ones. The plants grown in this way all have the same good qualities, which is very helpful for large-scale commercial cultivation (Asthana et al., 2011; Srinivas et al., 2014).

4 Optimization of Rapid Dissemination Technology

4.1 Key Factors affecting rapid spread

The quality of the nutrient soil and environment used for seedling cultivation has a significant impact on the rapid reproduction of young soapberry seedlings. In the commonly used MS nutrient soil, adding growth-promoting agents such as 6-benzylaminopine can make seedlings grow more easily. For instance, when four-sided saponin seedlings were placed in the nutrient soil treated with pesticides, approximately 97.22% could regrow well (Asthana et al., 2011). In addition, environmental conditions such as temperature and humidity are also very important. When the seedlings cultivated by this method are transplanted into the soil, 90% can survive (Asthana et al., 2011).

Treating seeds before sowing will also greatly affect seed germination and seedling growth. Studies have found that soaking seeds in hot water for 10 seconds is better than other treatment methods. The seeds germinate more easily and the seedlings are more likely to survive after being planted in the field (Haider et al., 2016). This indicates that choosing the right nutrient soil and properly handling seeds are the keys to improving the reproductive effect.

4.2 Techniques for promoting growth and improving the quality of seedlings

People use methods such as rapid cultivation and cutting propagation to make the young soapberry seedlings grow faster and of better quality. When using the rapid cultivation method, select the appropriate part of the seedlings, first place them in the liquid nutrient soil with indole-3-butyric acid added, and then transfer them to the solid nutrient soil. In this way, the seedlings can grow many and good roots (Asthana et al., 2011). In this way, the new seedlings grow the same as the original ones and have a well-developed root system.

Cutting propagation has found the best conditions for the branches to take root through many experiments. More than 92.3% of the tips of soapberry branches treated with rooting powder could grow roots (Yang, 2010). These methods can not only make the seedlings grow faster, but also make them stronger and have a higher survival rate.

4.3 Biotechnology innovation

New biotechnologies such as altering plant genes and using growth agents are crucial for optimizing the reproduction of soap pods. When artificially cultivating seedlings, the use of growth agents such as 6-benzylaminopine and indole-3-butyric acid can make the stems and roots of seedlings grow better (Asthana et al., 2011; Ji, 2013). These chemicals can help seedlings grow new tissues and sprout new buds, which is the key to the success of artificial cultivation (Zhou et al., 2012).

Although there are not many studies on soap pods using genetic modification technology at present, it may enable soap pods to reproduce faster and be more resistant to diseases in the future. It was found through detection that the seedlings propagated by the rapid cultivation method have the same genes as the original seedlings and can retain the original good characteristics (Asthana et al., 2011).

4.4 Successful cases and case studies on optimizing communication technologies

Many research examples have proved that the optimized propagation method is very effective in the cultivation of soap pods. For instance, the rapid cultivation method of the three-leaf soapberry can not only enable more new buds to grow and the roots to grow well, but also ensure that the new seedlings are the same as the original ones, suitable for large-scale planting (Asthana et al., 2011). It can be used in pharmaceuticals and also for further research.

There is another successful example. Soaking the acacia seeds in hot water increases the germination rate of the seeds, and the seedlings grow well. Eventually, they can all take root steadily in the ground (Haider et al., 2016). These examples illustrate that the optimized propagation method is very useful and can provide good ideas for future research and large-scale planting.

5 Resistance of Soapberry Seedlings to Abiotic Stress

5.1 Types of abiotic stress

Like many other plants, young soapberry seedlings also encounter various external environments that are unfavorable for their growth, such as drought and water shortage, excessive soil salinity, and fluctuating temperatures. During drought, there is insufficient water, and water is particularly important for the growth processes such as photosynthesis and nutrient transportation of seedlings. Water shortage will affect the growth and even survival of seedlings (Jisha and Puthur, 2015). Too much salt in the soil makes it difficult for seedlings to absorb water and nutrients and can also cause poisoning. This is salt stress (Singh and Kumar, 2021). Excessive temperature changes, whether extremely hot or cold, can interfere with the normal operation and internal activities of seedlings' cells, leading to poor growth of the seedlings (Qamar et al., 2022).

These unfavorable external environments can harm the growth process of young soapberry seedlings. For instance, drought and high soil salinity can cause oxidative damage to seedlings, just like having 'bad things" in the body, which can destroy the fat, protein and genetic material in the cells (Jisha and Puthur, 2015; Singh and Kumar, 2021). Large fluctuations in temperature can also be unbearable for seedlings, affecting the stability and normal functions of cells (Qamar et al., 2022).

5.2 Resistance mechanisms of *Quercus* plants

The young soapberry seedlings gradually developed some ways to cope with adverse environments. One of the important methods is to accumulate substances that can regulate the state of cells, such as proline and betaine. These substances can enable cells to maintain a normal state in adverse environments and protect the cell structure (Jisha and Puthur, 2015). In addition, seedlings will also activate their own "defense forces". Substances such as superoxide dismutase and catalase can eliminate the "bad things" in cells and reduce damage (Singh and Kumar, 2021).

Another key approach is to alter one's own growth process, such as enhancing the efficiency of photosynthesis and making more rational use of water, so that it can grow even in times of water shortage (Jisha and Puthur, 2015). When encountering a bad environment, certain genes in seedlings will "activate" and produce related proteins to counteract these stresses (Singh and Kumar, 2021).

5.3 Methods for enhancing abiotic resilience

To make the young soapberry seedlings more resistant to adverse external environments, the following methods can be used. Selecting those seedlings that are naturally highly resilient and then cultivating their offspring can gradually lead to the development of more stress-resistant varieties. Select individuals with good tolerance, and the entire population can become stronger (Puentes et al., 2018). Finding plants with strong stress resistance and using them for propagation can fundamentally enhance the stress tolerance (Singh and Kumar, 2021).

Another approach is to "assist" at the seed stage by treating the seeds with substances that can promote growth or protect cells, enabling the seedlings to better cope with harsh environments. For instance, treating seeds with β -amn-butyric acid can make seedlings more drought-tolerant and salt-tolerant because it can enhance the physiological and internal responses of seedlings (Jisha and Puthur, 2015; Qamar et al., 2022).

5.4 The influence of optimized propagation on stress resilience

The improved propagation methods, such as rapid cultivation and cutting propagation, have a significant impact on the stress resistance of soapberry seedlings. Rapid cultivation can quickly produce seedlings with the same genes and no diseases, which is crucial for maintaining the excellent characteristics of seedlings and enhancing their stress resistance (Asthana et al., 2011). When propagating, using specific growth agents can enable the root systems of seedlings to grow better, adapt to the environment more quickly and become stronger (Yang, 2010).

Moreover, a good propagation method can also pass on the characteristic of stress resistance to more seedlings. By selecting individuals with strong stress resistance for reproduction, varieties that are more adaptable to the environment can be cultivated. In this way, saponin seedlings are more likely to survive and grow in adverse environments, which is also conducive to the long-term cultivation of this plant with high economic value (Yang, 2010; Asthana et al., 2011).

6 Resistance of Soapberry Seedlings to Biological Stress

6.1 Common diseases and pests affecting *Pinus* plants

Like many crops grown in gardens, young soapberry seedlings are vulnerable to various pests and diseases, such as fungi, bacteria, viruses, and nematodes. These harmful substances can seriously affect the growth and development of seedlings, making them grow poorly, have low yields, and also limit the places where they can grow (Xu et al., 2022). If acacia is always planted in the same plot of land, the diseases in the soil will become more serious, greatly affecting the yield and quality of acacia (Xu et al., 2022).

Especially root-knot nematodes and some fungi pose a great threat to young soapberry seedlings. They can make the seedlings listless and even cause a large number of deaths. Therefore, some useful methods have to be thought of to reduce the harm they cause (Xu et al., 2022; Wu and Zhang, 2024).

6.2 Biological resistance mechanism

The young soapberry seedlings themselves have some ways to resist pests and diseases. One of them can produce some substances, which can drive away leaf-eating insects and also inhibit the growth of bacteria (Puentes et al., 2018). In addition, the genes of the seedlings themselves are also crucial. Just like different seedlings have different resistance abilities when facing insect bites, this is the role of genes (Puentes et al., 2018).

Cultivating soapberry seedlings with special seedling raising methods can make them more resistant to pests. Compared with the seedlings cultivated by ordinary methods, the seedlings cultivated by this method are attacked by pests less frequently and suffer less damage, indicating that this seedling cultivation method can enable the seedlings to activate the “defense mechanism” and enhance their ability to resist pests and diseases (Puentes et al., 2018).

6.3 Strategies for enhancing resistance to pests and diseases

To make the young soapberry seedlings more resistant to pests and diseases, you can try these methods. By means of biological control, such as introducing beneficial microorganisms or releasing some animals that specifically feed on pests, the number of pests can be controlled and the occurrence of diseases can be reduced without using pesticides (Xu et al., 2022). There are also comprehensive control methods, which combine improving planting conditions, biological control and the use of pesticides when necessary, and can comprehensively solve the problems of pests and diseases (Xu et al., 2022).

It is also a good approach to cultivate soapberry varieties that are resistant to pests and diseases through genetic modification and breeding. Identifying the genes for disease and pest resistance and then transferring them to newly cultivated varieties can make the young soapberry seedlings inherently more resistant to diseases and pests (Xu et al., 2022). If these methods are combined with modern biotechnology, the soapberry pods can better resist pests and diseases.

7 Current Challenges and Gaps in Research

7.1 Issues in scaling up fast propagation techniques for large-scale cultivation

One major challenge in promoting the rapid propagation technology of soapberry seedlings on a large scale is to adjust the indoor cultivation environment so that a large number of seedlings can be raised quickly and well, and each one is similar. Although there are already rapid cultivation methods, such as the technology for cultivating trilingual acacia, these methods require precise control of the dosage of growth-promoting agents and environmental conditions, and it is difficult to maintain stability all the time during large-scale cultivation (Asthana et al., 2011). During the cultivation process, growth agents of specific concentrations are used and the cultivated seedling parts need to be frequently transferred. All these make large-scale promotion difficult (Asthana et al., 2011).

Besides, not many of the seedlings that have been cultivated indoors can survive after being transplanted into the soil. Although the survival rate is very high when cultivated indoors, for instance, the three-leaf soapberry can reach 90%, when grown outdoors, due to factors such as soil quality and weather changes, the survival rate will

drop significantly (Asthana et al., 2011). This indicates that in-depth research is still needed on how to enable seedlings to better adapt to outdoor environments and develop a more robust cultivation system that can withstand natural changes.

7.2 Understand the limitations of the genetic mechanism of resistance

We still do not have a sufficient understanding of the genetic principles behind the stress resistance of acacia plants. Although through technical testing, it can be confirmed that the seedlings cultivated indoors have the same genes as the original plants, this cannot tell us exactly which genes give the plants the ability to resist stress (Asthana et al., 2011). Understanding these genes is particularly important for cultivating more resilient saponin seedling varieties.

Moreover, most of the current research focuses on how plants reproduce and how genes remain stable, without much study on the genetic roots of plants' resistance to pests, diseases and harsh environments.Don't understand these, it is very difficult for us to cultivate improved varieties that can grow well in various environments. Future research should aim to identify and study the genes that enable plants to withstand adverse conditions, so that young soapberry seedlings can grow better in different environments (Zhao, 2024).

7.3 Challenges in field application and adaptation to different ecological environments

When planting the cultivated young soapberry seedlings into the field, many problems will be encountered, especially how to make them adapt to different natural environments. Seedlings that are successfully cultivated indoors may not survive well when planted outdoors. For instance, if *Sapindus mugum* seedlings are transplanted into flowerpots, even if the soil in the flowerpots is treated, the survival rate is not high (Singh et al., 2010). This indicates that the cultivation methods need to be improved to enable the seedlings to make a better transition from indoors to outdoors.

The researchers are not quite clear about what acacia seedlings need in different environments and how they will react to stress, it is very difficult for them to adapt to different environments. Although some cultivation methods have been optimized for specific situations, such as cultivating with the tips of 15-centimeter-long *Sapindus* mugwort branches, these methods may not be applicable in other environments (Yang, 2010). The next research aims to develop flexible cultivation techniques and figure out what kind of environment different acacia tree species prefer, so that they can grow well in the fields and achieve large-scale planting.

8 Future Directions

Advanced genetic modification technologies, such as CRISPR, can significantly enhance the disease resistance and reproductive capacity of young soapberry seedlings. These technologies can "operate on" the genes of the soapberry, making them more resistant to diseases and enabling them to grow better in different environments. Take the three-leaf soap pod for example. Its own genes are relatively stable, which lays a good foundation for future research on genetic modification. With the help of advanced genetic modification technology, can identify and modify the genes that affect the tolerance and growth rate of the soapberry. In this way, perhaps we can cultivate stronger and more adaptable soapberry varieties.

Precise techniques for growing crops and environmental monitoring methods are particularly crucial for optimizing the propagation of soapberry seedlings. These techniques can accurately control soil moisture, the amount of nutrients and the temperature level, which are very important for the successful reproduction of soap pods. Previously, when specific research methods were used to test the propagation of soap pods by cuttings, it was found that adjusting the environmental conditions could make soap pods take root more easily and survive better. If the tools for precisely growing crops are put to use, the growers can reproduce the soapberry pods more efficiently, make the seedlings grow healthier and have a higher yield.

The collaboration among university research institutes, enterprises and farmers is indispensable for the sustainable cultivation of soap pods. When schools and research institutes conduct research, they can discover new knowledge and come up with new technologies. For instance, the methods for cultivating soapberries indoors that have been

developed can all be applied to enterprise production and agricultural planting. Enterprises can turn these technologies into products and expand the production scale. Meanwhile, farmers can, based on their actual planting experience, put forward improvement suggestions to make these technologies more in line with actual needs. When everyone works together, sustainable planting methods can be found, benefiting all parties.

9 Concluding Remarks

It's gained a lot in the research on how to make the soapberry seedlings reproduce faster. A good way to cultivate the three-leaf soapberry indoors has been found. By adding the agent BAP to the commonly used MS nutrient soil and using the 4-week-old seedling part for cultivation, almost 97.22% of them can regrow well. After further treatment with IBA agent, 91.67% of them could grow roots smoothly. Similarly, through the cutting propagation experiment, it was found that by applying ABT rooting powder to the tips of 15-centimeter-long *Sapindus* mugwort branches, 92.3% of the branches could grow roots, thus finding the most suitable propagation conditions for it. These methods not only accelerate the reproduction rate but also ensure that the newly grown soapberry seedlings are as good as the original varieties. After testing, it was found that the seedlings obtained by the rapid cultivation method have no genetic changes.

To fully leverage the ecological and economic benefits of acacia trees, it is necessary to constantly study how to grow them well. The reliable propagation methods have developed, can ensure that the acacia tree keeps producing. After all, the acacia tree can be used not only as medicine but also for other purposes, which is of great value. If continue to improve these planting techniques, the acacia tree will be able to play a greater role in pharmaceuticals and environmental improvement, which is helpful for protecting biodiversity and developing the economy.

The acacia tree, as it can be used as medicine and is beneficial to the ecology, is sure to become an important sustainable resource in the future. Nowadays, with the advancement of propagation technology, it will be possible to plant soapberry trees on a large scale in the future. This will not only provide raw materials for pharmaceutical factories but also contribute to ecological protection. As research deepens, the role that acacia trees can play in sustainable agriculture and environmental protection will become increasingly significant, providing useful resources for achieving the global sustainable Development goals.

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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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