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Progress in Research on Propagation Technology of *Machilus* Nees

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Abstract *Machilus* spp. are mostly valuable timber and economic tree species, widely used in many fields such as artificial afforestation, landscaping, furniture and building materials, chemicals and medicine. In this article, the distribution, uses and domestic resources of *Machilus* Nees are summarized. Focus is placed on the analysis of propagation methods, including seed propagation, cutting propagation and tissue culture propagation. The research status and existing problems are summarized, and the future research focus on the propagation technology of *Machilus* Nees is prospected, with a view to provide theoretical references for the efficient breeding of high-quality seedlings and comprehensive promotion and application of *Machilus* spp. and contribute to the conservation and sustainable use of wild resources.

Key words *Machilus* Nees, Seed reproduction, Cutting, Tissue culture

1 Introduction

Machilus Nees (Lauraceae: Trib. Perseae Mez.) is an evergreen basal angiosperm, tree or shrub, widely distributed. There are about 100 species worldwide, most of which are distributed in tropical and subtropical regions of Southeast Asia and East Asia. China is the center of its distribution. Currently, 68 species and 3 variants of *Machilus* Nees have been recorded. They are concentrated in the provinces and autonomous regions south of the Yangtze River, especially in Yunnan and South China[1-4].

Machilus Nees is a major economic forest tree in southern China. Most species are representatives of tropical and subtropical forests[5-6], and are evergreen and beautiful in shape. Some species can secrete phytobacterin and purify the air, and are ideal landscaping tree species[7]. The wood has straight texture, and is resistant to corrosion and insects. It has been widely used in the production of precious furniture, high-end buildings and ships since ancient times[8-9]. The bark of many species can be made into brown dye. The bark and leaves can be ground into powder for drinking water purification agent or various aromatherapy agents. The branches, leaves and fruits can be used to extract aromatic oil. The seed oil can be made into soap or processed into lubricating oil[10]. In addition, some species of *Machilus* Nees are used as herbal medicine in China, with significant biological activity, such as antibacterial, analgesic, anti-inflammatory and anti-infective. They are mainly used for the treatment of bloating, diarrhea, sprains, *etc.*, and have high medicinal value[11]. As *Machilus* spp. are valuable timber and economic tree species, coupled with not getting enough attention, a large area of natural vegetation of *Machilus* spp. has been destroyed by human activities. Natural forest resources of *Machilus* Nees are facing depletion. At present, the number of natural forests of *Machilus* Nees that can be verified is very limited. Some species have been classified as national second-level protected tree species[12-14]. *Machilus* spp. usually grow in a humid and rainy environment. Their seeds are mostly fleshy, and are perishable after landing. Their seedlings grow slowly, leading to poor natural propagation of some tree species[15-16]. In addition, *Machilus* spp. have low resistance to the environment, and their regeneration ability in the natural environment is not high. Some species can only survive in specific areas and environments[17]. In the research on the physiological and ecological characteristics of seeds of secondary forest tree species in Hong Kong, Zhuang Xueying *et al.* [18] found that the seeds of *M. breviflora* and *M. velutina* could not sprout naturally under the forest, so even without human destruction, the advantage of *Machilus* Nees in the existing secondary forest will be replaced by other tree species that are more shade-tolerant and more fertile. At present, artificial propagation is the most effective method for large-scale breeding of *Machilus* seedlings and protection of the resources. In recent years, researches on the propagation technology of *Machilus* Nees have been increasing, involving seed propagation, cutting propagation and tissue culture. This article intends to conduct a systematic review of the research status of the breeding technology of *Machilus* Nees, with a view to provide references for efficient breeding of high-quality seedlings of *Machilus* Nees and contribute to the conservation and sustainable use of wild resources.

2 Sowing and seedling raising of *Machilus* Nees

Seed propagation is currently the most important breeding method of *Machilus* Nees. By consulting relevant literature, the seed germination rate of several species of *Machilus* Nees under different optimal treatments is sorted out. As shown in Table 1, under proper treatment, the seed germination rate of *Machilus* Nees is between 70% and 100%, and the overall difference is small. Among the 8 species of *Machilus* Nees, the germination rate of

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M. microcarpa[19] and *M. thunbergii*[20] is relatively low, both around 70%; and after being soaked with 10 mg/L of gibberellin (GA_3) for 24 h, the germination rate of *M. breviflora*[21] could reach 100%. Zhang Dongsheng *et al.*[22] studied the effect of plant growth regulators on the germination of *M. pauhoi* seeds, and found that in the treatment of soaking with 100 mg/L of naphthaleneacetic acid (NAA) for 24 h, the germination rate was the highest (94.44%), but the germination index was the lowest, the average germination days were the longest, and the average seedling height was the smallest; and after treatment with 50 mg/L of GA_3, the germination rate was 88.89%, slightly lower than that in the NAA treatment, but the seed germination index, germination days and average seedling height were significantly better than other treatments. In addition, for *M. dauzhenensis*[23], GA_3 is also the best plant growth regulator for treating seeds, with a germination rate between 80% and 90%. Therefore, for most *Machilus* species, soaking seeds with GA_3 may promote seed germination and increase the germination rate. If the seeds were washed and dried in the shade before sowing, the germination rate of *M. chlnensis* can reach more than 90%; the germination rate of newly-harvested seeds is only about 70%; and fully dried seeds have no germination capacity[24]. For *M. yumnunensis*[25], *M. microcarpa*[19], *M. longipedicellata*[26] and *M. thunbergii*[27], pretreatment with wet sand or wet towels before sowing can greatly improve the germination rate and seedling survival rate.

Table 1 Effects of different treatments on seed germination rate of *Machilus* spp.

Code	Species	Treatment method	Germination rate // %
1	*M. breviflora*	Soaking in 10 mg/L of GA_3 for 24 h	100
2	*M. yumnunensis*	Wet storage with sand (river sand: seed = 3:1)	85 – 90
3	*M. microcarpa*	Nursing with wet towel and sowing after germination	70
4	*M. chlnensis*	Cleaning and drying in shade for sowing	90
5	*M. pauhoi*	Soaking in 100 mg/L of NAA for 24 h	94.44
6	*M. dauzhenensis*	Soaking in 200 mg/L of GA_3 for 24 h	80 – 90
7	*M. longipedicellata*	Storing with sand and sowing in February – March next year	94.1
8	*M. thunbergii*	Storing in wet sand and sowing after more than half of the seeds germinate	70

Note: Treatment listed is that with the highest germination rate.

3 Cutting propagation of *Machilus* Ness

Although seed propagation is currently the main method for the reproduction of *Machilus* Nees, its seedling formation is slow, and variation and differentiation are serious. Some high quality individual plants gradually lose their excellent traits due to long-term seed propagation. What's more, seed reproduction is susceptible to yielding difference between years. Cutting propagation can better preserve the excellent traits of the female parent[27 – 28]. Therefore, research on cutting propagation technology of *Machilus* Nees is of great significance for the artificial cultivation and high-quality germplasm breeding of this genus. There are many factors that affect the survival of cuttings of *Machilus* Nees. The current research direction mainly focuses on the influencing factors of rooting of cuttings, such as cutting specifications, cutting processing, cutting time and substrate. As shown in Table 2, among the 8 species of *Machilus* Nees, the highest survival rate of cuttings under optimal cutting conditions was 89.18%, and the lowest was only 48.89%, which were quite different.

3.1 Specifications of cuttings Specifications of cuttings, such as degree of lignification and length, all have a significant effect on the survival rate of cuttings[37]. As shown in Table 2, for most species of *Machilus* Nees, semi-lignified branches of the current year, 10 – 12 cm long, are more suitable for cutting. In the research on cutting technology of *M. velutina* and *M. versicolora*, cuttings of different lignification degrees and lengths were designed for cutting. The results show that the two species of *Machilus* Nees were more likely to take roots with cuttings of 12 cm in length, and the rooting rates among different specifications of cuttings were in the order as semi-lignified green branch > semi-lignified tender branch > fully lignified hard branch. Among them, the rooting rate of semi-lignified green branches of *M. velutina* was 88.5%, and that of *M. versicolora* was 83.2%; and the rooting rate of fully lignified hard branches was the lowest, 74.2% for *M. velutina* and 49.9% for *M. versicolora*[31, 34]. When the lignification degree of cuttings increases, their metabolism capacity will be weakened, and the content of rooting inhibitors such as tannins and phenols in plant organs will also increase, making it more difficult to activate the root primordia and other tissues during cutting, thus producing an impact on the rooting of cuttings[38 – 39]. Whether cutting carries apical bud will also affect the cutting effect[40 – 41]. However, in the research on cutting technology of *M. pauhoi*, Pan Hanqi[42] found the rooting rate of cuttings with or without apical buds was not significantly different. The main reason is that *M. pauhoi* has good budding ability, and cuttings usually sprout first and then root, so whether or not there is a top bud does not affect the cutting effect. Therefore, the rooting rate and rooting time were not significantly different.

3.2 Processing of cuttings Cutting processing mainly includes the use of plant growth regulators, as well as etiolated treatment and leaves and incision treatment. Plant growth regulators are synthetic compounds that can promote or retard plant growth. Treating cuttings with them is the main technical method to promote the rooting of the cuttings[43]. The current research direction mainly focuses on the influence of species, concentration and treatment time on the survival rate of cuttings of *Machilus* Nees. After the base of *M. breviflora* cuttings was soaked with ABT rooting powder

(800 mg/L) for 30 min, the comprehensive cutting effect was the best, with a rooting rate of 48.89%[36]; and after the cuttings of *M. litseifolia* were treated with 100 mg/kg IAA or 100 mg/kg ABT-1# for 12 h, the survival rate of the cuttings was as high as 89.18%[33]. For some species, single growth regulator is often difficult to induce rooting. Mixing different growth regulators will help rooting[44]. Sun Ang[32] studied the effects of three hormones, IBA, NAA, and IAA, on the rooting of *M. longipedicellata* cuttings. The results show that in the combination of 750 mg/L IAA + 400 mg/L IBA, the rooting rate, average number of primary roots, diameter of primary roots and root length were 85.5%, 3.5 strips/plant, 1.58 mm and 13.5 cm, respectively. It was the optimal treatment combination for comprehensive evaluation of rooting traits. Etiolated treatment can be applied to cuttings of species with low rooting rate[38]. After etiolated treatment, the average survival rate and rooting rate of cuttings of *M. pauhoi* were 56.3% and 43.5%, which were 25% and 22.1% higher than those of cuttings without etiolated treatment, respectively. With equal leaf area, the cutting effect of *M. pauhoi* cuttings with two half leaf was better than that of cuttings with one entire leaf, and the survival rate and rooting rate were 7.9% and 5.4% higher, respectively[45].

Table 2 Research results of cutting propagation of *Machilus* Nees

Code	Species	Cutting treatment	Cutting time	Cutting specifications	Cutting substrate	Rooting rate//%	Reference
1	*M. pauhoi*	Treating cuttings with 100 mg/kg of indolebutyric acid (IBA)	April	Tender branch, spike length 7 – 10 cm, with axillary buds, with 2 – 3 half leaves	Muddy loess	56.96	Zhao Hongmei[29]
2	*M. chekiarcgensis*	Soaking base of cuttings with 100 mg/L of NAA for 24 h	Spring	Semi-lignized, ear length 8 – 10 cm, 1 to half leaves on top, apical bud	River sand	78.00	Lin Xiongping[30]
3	*M. velutina*	Dipping in rooting powder No. 1 (ABT-1#) with mass fraction of 650×10^{-6}	November	Semi-lignized tender branch, spike length of 12 cm	Red subsoil	88.50	Wu Yongjun[31]
4	*M. longipedicellata*	Dipping in 750 mg/L indoleacetic acid (IAA) + 400 mg/L IBA	–	Semi-lignified tender branch, spike length 10 cm long, with 2 to 3 half leaves, flat upper cut, wedge-shaped lower cut	Red soil	85.50	Sun Ang[32]
5	*M. litseifolia*	Soaking in 100 mg/kg of IAA or 100 mg/kg of ABT-1# for 12 h	February – July	Annual branch without leaves, spike length 10 – 12 cm	River sand	89.18	Chen Suiliang[33]
6	*M. versicolora*	Dipping in ABT-1# with mass fraction of 650×10^{-6}	March	Semi-lignified green branch, spike length 12 cm, oblique cut at base	50% red soil + 50% perlite	89.10	Zhang Haikun[34]
7	*M. Chlnensis*	–	December	Current-year new shoot with new buds, spike length 3 – 5 cm, with more than two axillary buds, with 2 – 3 half leaves and top bud	90% latosolic red soil + 10% burned soil	85.00	Yang Haidong[35]
8	*M. breviflora*	Treating base of cuttings with 800 mg/L of ABT for 30 min	June – September	Current-year semi-lignified branch, spike length 8 – 12 cm, oblique cut at the base, with 3 – 5 half leaves	Yellow sub soil + river sand + perlite (1:1:1)	48.89	Xu Yin[36]

Note: Treatment listed is that with the highest germination rate.

3.3 Cutting time and substrate Cutting time and substrate are the two main external factors that affect and restrict the rooting of cuttings. Lin Xiongping[30] studied the effects of different time and substrates on the survival rate of cuttings of *M. chekiarcgensis*, and the results show that there were significant differences in the survival rate of cuttings at different periods. The average cutting survival rate of *M. chekiarcgensis* reached 74% in spring, which was significantly higher than the survival rate of 10% in autumn; and the survival rate of cuttings in sand was higher than that in soil in the same period. In the cutting trial of *M. chlnensis*, Yang Haidong[35] found that the rooting rate of cuttings and the growth of seedlings differed significantly among different cutting time. Among them, the rooting rate of cuttings was the highest in winter (December), reaching 85%, and the seedling growth was the best; the rooting rate of cuttings was the lowest in spring, only 30%, and the seedling growth was not good; and there were significant differences in the rooting rates of *M. chlnensis* in three substrates, and the rooting rates ranked as 90% latosolic red soil + 10% burned soil (87%) > latosolic red soil (83%) > pure burned soil (71%). Using a four-factor, three-level orthogonal test, Tang Jianmin investigated the effects of four factors, substrate, growth hormone, hormone concentration and soaking time, on the rooting of *M. pauhoi* cuttings. The results show that substrate was the most important influencing factor on the rooting of *M. pauhoi* cuttings. The rooting rate of *M. pauhoi* cuttings differed significantly in the substrates composed of different proportions of muddy loess and fine sand. Among different substrates, the rooting rate of the cuttings in the substrate composed of 90% muddy loess and 10% fine sand was the highest (86.2%), while that in the substrate composed of 50% muddy loess and 50% fine sand was the lowest (52.8%)[42]. The results of the studies above indicate that the optimal cutting time of *Machilus* Nees varies among different species. Most species have the best cutting performance in substrates with good air permeability and a certain water retention capacity.

4 Tissue culture propagation of *Machilus* Nees

Plant tissue culture technology is a new asexual reproduction technology developed in recent decades. For the species of high economic and ornamental value of *Machilus* Nees, tissue culture is able to achieve rapid reproduction and retain excellent traits, and can be used for production throughout the year. However, there are few reports on the tissue culture propagation of *Machilus* Nees at home and abroad. Chen Yijia *et al.* [46] used different parts of the sterile seedlings of *M. pauhoi* as explants to carry out a tissue culture experiment. The results show that cotyledonary nodes were the most likely to induce adventitious buds, with the largest number of induced buds; and the regeneration ability of clumpy buds was weak. The more suitable propagation method was as follows: pre-culturing explants on MS + 6-BA 3.0 mg/L + IBA 0.01 mg/L + $AgNO_3$ 1.0 mg/L for 10 d and transferring explants to MS + 6-BA 3.0 mg/L + IBA 0.01 mg/L. The proliferation coefficient reached 3.52. Addition of $AgNO_3$ could significantly increase the proliferation coefficient. However, high concentration of $AgNO_3$ would cause malformation of the shoots. The optimal rooting medium was 1/2 MS + IBA 0.2 mg/L, in which the rooting rate could reach 91%. Chen Chun *et al.* [47] studied the tissue culture technology of *M. versicolora*. The results show that after treating the stem sections of *M. versicolora* with 75% alcohol for 15 s and then sterilizing them with 0.1% mercury for 10 min, the pollution rate of the explants was less than 22%, and their survival rate reached 60.0%. The best induction medium was MS (improved) + 6-BA 0.5 mg/L + NAA 0.1 mg/L + sucrose 30 g/L + ascorbic acid 1.0 g/L.

Wang Tingjin *et al.* [48] used lateral buds and top buds of *M. yumnunensis* as tissue culture material. The results show that the best substrate for callus formation in root sprouts was MS + 2,4-D 0.5 mg/L + BA 2mg/L + AC 0.3%; the best substrate for germination of lateral buds was MS + 2,4-D 0.1 mg/L - 1 + KT 2 mg/L + AC 0.3%; and the best substrate for germination of top bus was MS + NAA 1 mg/L + BA 1.5 mg/L + AC 0.3%. Factors such as source of explant, time of harvest and type and concentration of hormone were the main reasons for browning of tissue culture and affecting rapid reproduction of *M. yumnunensis*[49].

5 Conclusions and recommendations

5.1 Conclusions Among the three propagation methods for *Machilus* Nees, seed propagation is a relatively mature and widely used propagation method, and its average survival rate is also higher. But in the actual production process, if woody plants adopt this propagation method, problems such as long nursery time and difficulty in preserving some excellent properties still exist[50]. The selection of appropriate treatment methods can greatly improve the germination rate of *Machilus* seeds. For example, soaking seeds with the appropriate concentration of GA_3 can improve the germination rate of M. breviflora, *M. pauhoi* and *M. dauzhenensis*[21-23], which can provide a theoretical reference for the selection of other plant growth regulators for *Machilus* Nees. As the seed coats of most *Machilus* Nees plants are thick, sowing after pre-germination treatment can greatly improve the germination rate and seedling survival rate.

5.2 Recommendations *Machilus* Nees has broad application and development prospects. Most species have been applied and promoted in many fields such as artificial afforestation, landscaping, furniture and building materials, chemicals and medicine. In recent years, research on this genus has been increasing. However, the successful promotion of any plant is inseparable from its perfect reproduction technology system. At present, research on cutting propagation technology of *Machilus* Nees has not yet formed a relatively complete system. It mainly focuses on the factors that affect the survival rate of cuttings. A systematic study of the combination of micromorphological observation and macromorphological observation and the research on the physiological and biochemical mechanism of cutting rooting are particularly lacking. In order to enable the propagation quantity and quality of *Machilus* Nees germplasm resources adapt to new development needs, research in this area should be strengthened. It is suggested that in researching the rapid propagation technology of *Machilus* spp. suitable for production, the following aspects should be explored in depth: (i) rooting types and the number and distribution of root primordia; (ii) dynamic changes of plant nutrients and endogenous hormones during rooting; (iii) regulation of endogenous inhibitory substances; (iv) regulation of external environmental factors. From the perspective of propagation technology, tissue culture has the advantages that cannot be replaced by many other propagation methods, such as seed propagation and cutting propagation. This method can maintain stable good traits. It has the characteristics of less material collection, short seedling cycle and fast reproduction speed[51], but implementation is relatively difficult. If the problems in the tissue culture of *Machilus* Nees are resolved through research and this biotechnology is utilized fully, the large-scale and efficient breeding of *Machilus* spp. with great economic value will be greatly promoted.

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