Experimental Study on Cutting and Seedling Cultivation of *Ilex Latifolia*

Aifang Tian[1], Hecheng Zhang[1], Yanhong Zhang[2,*], Xinling Zhang[1]
[1]*Luohe Landscape and Greening Maintenance Center, Luohe, Henan, China*
[2]*Zhengzhou Zoo, Zhengzhou, Henan, China*
**Corresponding Author.*

Abstract: The growth rate of *Ilex latifolia* is slow and difficult to sow. This article explores the cutting propagation technology of *Ilex latifolia* Based on the ecological and growth characteristics of *Ilex latifolia*, experiments were conducted on rooting of hard branches of *Ilex latifolia* using different types and concentrations of plant growth regulators. The results showed that among the three plant growth regulators, ABT rooting powder, NAA, and IBA, ABT treatment at a concentration of 800 × 10^{-6}mg/ml was the most suitable for the propagation of *Ilex latifolia*, IBA treatment at a concentration of 50 × 10^{-6}mg/ml had better effects, and ABT treatment at a concentration of 300 × 10^{-6}mg/ml had the worst effects.

Keywords: *Ilex Latifolia*; Propagation Through Cuttings; ABT Rooting Powder; Naphthylacetic Acid; Indole Butyric Acid

1. Preface

Ilex latifolia Thunb is a large evergreen tree in the holly family, with a height of up to 20 meters and a diameter at breast height of 60 centimeters. It is completely hairless; The bark is gray black in color; Branches are thick and robust, with longitudinal edges and grooves, yellow brown or brown, smooth, with obvious raised, wide triangular or semi-circular leaf scars. Leaves grow on branches that are 1-3 years old, with thick leathery leaves that are oblong or ovate in shape. They are 8-19 (-28) centimeters long and 4.5-7.5 (-9) centimeters wide, with a blunt or short pointed tip and a circular or broadly wedge-shaped base. The edges have sparse serrations, and the tooth tips are black. The leaf surface is dark green and glossy, and the back is light green. The midrib is depressed on the leaf surface and raised on the back. There are 12-17 lateral veins on each side, which are obvious on the leaf surface but not obvious on the back; The petiole is thick, nearly cylindrical, 1.5-2.5 centimeters long, with a diameter of about 3 millimeters, slightly concave on the upper surface, and wrinkled on the back surface; The supporting leaves are extremely small, wide triangular, and sharply pointed. A false cone-shaped inflorescence composed of umbrella inflorescences grows in the leaf axils of biennial branches, without a total stem; The main axis is 1-2 centimeters long, with persistent round, tile shaped arranged scales at the base, and a membranous inner surface that is relatively large. Flowers light yellow green, 4 cardinal numbers. Male flowers: Each branch of the false cone-shaped inflorescence has 3-9 flowers, forming a clustered inflorescence with a total peduncle length of 2 millimeters; Bracts ovate or lanceolate, 5-7 millimeters long and 3-5 millimeters wide; The flower stem is 6-8 millimeters long, with 1-2 small bracteoles, triangular in shape; Calyx nearly cup-shaped, with a diameter of about 3.5 millimeters, 4 shallow lobes, and circular lobes; The corolla is radial, with a diameter of about 9 millimeters, and the petals are ovate oblong, about 3.5 millimeters long and 2.5 millimeters wide, with a fused base; The stamens are of equal length to the petals, and the anthers are ovate and elongated, twice the length of the filaments; The sterile ovary is nearly spherical, with a slightly four lobed stigma. Female flowers: Each branch of the inflorescence has 1-3 flowers, with a total peduncle length of about 2 millimeters. The peduncle of a single flower is 5-8 millimeters long and has 1-2 bracteoles; Calyx disc-shaped, with a diameter of about 3 millimeters; The corolla is upright, with a diameter of about 5 millimeters; Petals 4, ovate, approximately 3 millimeters long and 2 millimeters wide; The degenerated stamen is 1/3 of the petal length, and the aborted anthers are small and oval in shape; The ovary is ovoid, with a diameter of about 2 millimeters, and the stigma is

disc-shaped, with 4 lobes. The fruit is spherical in shape, with a diameter of about 7 millimeters. When mature, it is red in color. The persistent stigma is thin and disc-shaped, and the base of the calyx is disc-shaped, extending. The outer fruit skin is thick and smooth. Divided into 4 nuclei, with a contour of elongated elliptical shape, about 5 millimeters long and 2.5 millimeters wide, with irregular wrinkles and dust pits, obvious longitudinal ridges on the back, and endocarp bone. The flowering period is in April, and the fruiting period is from 9-10 [1].

Large leaved holly is produced in provinces and regions such as Jiangsu, Anhui, Zhejiang, Jiangxi, and Henan in China, and is mainly distributed in Japan abroad; Born in evergreen broad-leaved forests, shrubs, or bamboo forests on mountain slopes at an altitude of 250-1500 meters [2]. Slow growing, tolerant to shade and humidity, prefers warm and fertile sandy loam soil, and can be planted in places with an average annual temperature of not less than -10 ℃; It also has good soil and water conservation ability [3].

Large leaved holly wood can be used as fine wood material, and the bark can be used to extract tannin extract. The plant is beautiful and can be used as a garden greening tree species [4]. Kuding tea made from its leaves is rich in beneficial ingredients such as tannins, vitamins, proteins, inorganic salts, and various trace elements; Moreover, according to the Compendium of Materia Medica, *Ilex latifolia* has a bitter, astringent, and cold taste, and has the functions of clearing heat and detoxifying, dispersing wind, promoting blood circulation, and stopping bleeding [5]. According to the Pharmacopoeia of the People's Republic of China, long-term consumption of large leaf holly has good medical and health benefits for dizziness, tinnitus, premature graying of hair, chronic benzene poisoning, leukopenia, mountain sickness, and atomic radiation. It is known as "health tea", "beauty tea", "weight loss tea", and "longevity tea". With the improvement of people's living standards, consumers' pursuit of "pollution-free, safe, high-quality, and healthy" food has become a trend [6]. *Ilex latifolia*, as a green food with high nutritional and health value, meets the demand of consumers for zero pollution food and has good development prospects [7]. *Ilex latifolia* is mostly wild and has not been artificially domesticated. Under natural conditions, its embryo and surrounding tissues develop inconsistently, with a long post maturity period, also known as the "two-year seed". Therefore, seed reproduction is extremely difficult to survive [8-12], which seriously restricts the development and utilization of *Ilex latifolia* seedlings in production. In order to meet the high demand for large leaf holly seedlings in the context of returning farmland to forests and the market economy, and to promote the rooting and survival rates of cuttings, three rooting agents, ABT, NAA, and IBA, were used to conduct experimental research on the survival and rooting rates of large leaf holly cuttings.

2. Materials and Methods

2.1 Overview of the Experimental Site

Luohe City is located in the central southern part of Henan Province, with geographical coordinates of 113 ° 27 ′ -114 ° 16 ′ E longitude and 33 ° 24 ′ -33 ° 59 ′ N. Located on the southern edge of the warm temperate zone, it belongs to the warm transitional monsoon climate, with alternating cold and hot temperatures throughout the year and distinct four seasons. The climate is characterized by cold winters with less rain and snow, hot summers with concentrated rainfall, cool autumns with long sunshine, and dry and windy springs. The average annual temperature throughout the city is 14.6 ℃. The hottest month is July, with an average annual temperature of 27.4-27.7 ℃. The coldest month is January, with an average temperature of 0.5-0.7 ℃. The average annual precipitation is moderate, but the interannual variation of precipitation is large, and the spatial and temporal distribution within the year is uneven. Generally, there is less precipitation in winter and spring, more precipitation in summer and autumn, and the rainfall is mostly concentrated in July and August [13].

2.2 Test Materials

The cutting materials of *Ilex latifolia* come from Changzhuyuan Township in Shangcheng County and Shawo Town in Xinxian County, Henan Province. Plant growth regulators include ABT rooting powder (ABT); Naphthylacetic acid (NAA); Indole butyric acid (IBA)and water.

2.3 Test Methods

Prepare solutions of ABT and NAA with mass concentrations of 100×10^{-6}、300×10^{-6}、500×10^{-6}、800×10^{-6}mg/ml, and prepare solutions of IBA with mass concentrations of 50×10^{-6}、100×10^{-6}、300×10^{-6}、500×10^{-6}mg/ml for later use, while using clean water as a control treatment. The cutting materials are all made of hard branches, and the cuttings contain three full buds. Soak the selected cuttings in the rooting agent solution for the time shown in Table 1. Disinfect the soil with a 500 fold carbendazim solution one week before cutting to improve the survival rate of *Ilex latifolia* cuttings and prevent adverse effects of soil environment on the experiment. The investigation of rooting rate was conducted nearly 40 days after cutting. The cutting time is selected in April, and the rooting rate survey will be conducted in May.

Table 1. Time for Soaking Cuttings with Different Concentrations and Rooting Agents(h)

Concentration (mg/ml)	50	100	300	500	800	CK
ABT		4	2	1	0.5	0.5
NAA		12	6	4	3	0.5
IBA	24	16	4	3		0.5

3. Results and Analysis

3.1 The Effect of the Same Concentration of Rooting Agent on the Rooting of *Ilex Latifolia* Cuttings

From Table 2, it can be seen that when the mass concentration of the three rooting agents is 100×10^{-6}mg/ml, the IBA treatment has a better rooting rate for large leaf holly, with a survival rate of 78.2%. The rooting rate of large leaf holly treated with IBA is 67.2% higher than that treated with ABT, 40.2% higher than NAA, while the control treatment is only 30%. From this, it can be seen that under the concentration of 100×10^{-6}mg/ml of these three drugs, IBA has the best rooting effect on *Ilex latifolia*, which is nearly 58.2% higher than the control. When the concentration of three rooting agents was 300×10^{-6}mg/ml, IBA treatment had a better rooting rate for large leaf holly, with a survival rate of up to 85%. IBA treatment had a rooting rate 14% higher than NAA treatment and 76% higher than ABT treatment. From this, it can be seen that at a concentration of 300×10^{-6}mg/ml, IBA has a better rooting effect on *Ilex latifolia*, while NAA has a more prominent effect. However, the rooting rate of ABT is only 9%, which is not effective. When the concentration of three rooting agents was 500×10^{-6}mg/ml, IBA treatment still had the best rooting rate for large leaf holly, with a survival rate of up to 77%. IBA treatment had 8% and 11% higher root rates than NAA and ABT treatment, while at this concentration, there was almost no difference between ABT treatment and NAA treatment. From this, it can be seen that at a concentration of 500×10^{-6}mg/ml, IBA has the best rooting effect on *Ilex latifolia*, while ABT and NAA have almost identical effects, and the effects of these three growth regulators are all more ideal than the control.

Table 2. Effect of Rooting Agent of the Same Concentration on the Survival Rate of Cuttings (%)

Concentration (mg/ml)	ABT	NAA	IBA	CK
100	11	38	78.2	30
300	9	71	85	30
500	66	69	77	30

3.2 The Effect of Different Concentrations of the Same Rooting Agent on the Rooting of *Ilex Latifolia* Cuttings

From Table 3, it can be seen that when the concentration of ABT increased from 100×10^{-6}mg/ml to 300×10^{-6}mg/ml, the rooting rate of *Ilex latifolia* decreased by 2%; When the mass concentration was increased to 500×10^{-6}mg/ml, the rooting rate of *Ilex latifolia* increased significantly, with a 52% increase compared to the rooting rate at a mass concentration of 100×10^{-6}mg/ml and a 54% increase compared to the rooting rate at a mass concentration of 300×10^{-6}mg/ml; When the mass concentration is increased to 800×10^{-6}mg/ml, the rooting rate of *Ilex latifolia* reaches 88%. It can be concluded that the best rooting effect of *Ilex latifolia* is achieved when treated with ABT at a concentration of 800×10^{-6}mg/ml.

Table 3. The Effect of Different Depth Rooting Agents on the Rooting Rate of Cuttings (%)

Concentration (mg/ml)	50	100	300	500	800
ABT		13	11	65	88
NAA		46	72	64	63
IBA	85.7	79.5	84	78.2	

When the concentration of NAA increased from 100×10^{-6}mg/ml to 300×10^{-6}mg/ml, the rooting

rate of *Ilex latifolia* increased by 26%; When the mass concentration was increased to 500 × 10^{-6}mg/ml, the rooting rate of *Ilex latifolia* actually decreased by 8%; When the mass concentration is increased to 800 × 10^{-6}mg/ml, the rooting rate of *Ilex latifolia* further decreases. Therefore, the optimal concentration for NAA treatment is 300 × 10^{-6}mg/ml, but although the rooting rate is the highest at this time, it is only 72%. From an economic value perspective, it does not meet the current requirements for seedling cultivation of *Ilex latifolia*. When the concentration of IBA is 50 × 10^{-6}mg/ml, the rooting rate is the highest, reaching 85.7%; When the mass concentration increased to 100 × 10^{-6}mg/ml, the rooting rate actually decreased by 6.2%; When the mass concentration increased to 300 × 10^{-6}mg/ml, the rooting rate began to increase again, which was 4.5% higher than the mass concentration of 100 × 10^{-6}mg/ml, but still 1.7% lower than the mass concentration of 50 × 10^{-6}mg/ml; When the mass concentration increased to 500 × 10^{-6}mg/ml, the rooting rate began to decrease again, a decrease of 5.8% compared to the mass concentration of 300 × 10^{-6}mg/ml. It can be concluded that the rooting effect of *Ilex latifolia* is better when treated with IBA at a concentration of 50 × 10^{-6}mg/ml.

4. Discussion

The results of this experiment indicate that ABT powder has a good promoting effect on the rooting of *Ilex latifolia* cuttings, compared with Liu Dechao [14] et al. and Chen Xiaochou [15] The consistent research results on the rooting effect of cuttings from trees such as white fruited peach and purple flowered laughing indicate that ABT 1 rooting powder may be related to tree species The endogenous growth hormone regulators synthesized by the organism itself have a synergistic promoting effect, accelerating the progress of multiple metabolic processes such as protein hydrolysis and glucose metabolism Cheng promotes the formation and growth of root radicals [16], but the specific regulatory mechanism requires further research on the metabolic processes of *Ilex latifolia*.

5. Conclusion

When treated with IBA at a concentration of 50 × 10^{-6}mg/ml, the rooting effect of *Ilex latifolia* was better, with a rooting rate of 85.7%; When treated with ABT at a concentration of 800 × 10^{-6}mg/ml, the rooting effect of large leaf holly is the best, with a rooting rate of up to 88%. However, the rooting effect at low concentrations of ABT is not ideal compared to other growth regulators and other concentrations of drugs. It is recommended not to use low concentrations of ABT in the propagation of large leaf holly. From practice, it has been found that ABT rooting powder is a good rooting agent in the process of cutting *Ilex latifolia*, and IBA also has excellent effects, which is worth applying and promoting in production.

Reference

[1] Chinese Plant Compilation Committee, Flora of China, Volume 45 (2) (1999): 107
[2] Hu Qingdong, Zhou Biyong. The Utilization Value and Sowing and Seedling Technology of *Ilex latifolia* Modern Agricultural Technology, 2009 (5): 2
[3] Wan Guangzhong Cultivation Techniques of *Ilex latifolia* Anhui Forestry, 2007 (1): 26-26
[4] Steward Liu Kuding non tea has miraculous effects Guizhou Tea, 1996 (3): 38
[5] Chen Zhihui, Yang Ruxing, etc Chinese *Ilex latifolia* Tea Science and Technology, 1999 (3); 37-38
[6] Fu Jianyu, Liu Dehua, etc Research on Short Spike Cutting Seedling Technology of *Ilex latifolia* Tree Journal of Hunan Agricultural University, 1994, 20 (2): 118
[7] Chen Zhangzhou Morphological characteristics, ecological habits, and growth characteristics of *Ilex latifolia* species Sericulture Tea Newsletter, 1997, 88 (2): 38-39
[8] Li Jihua, The principle and application of cuttings. Shanghai: Shanghai Science and Technology Press, 1987, 23-25
[9] Praise strictness; Yang Zhiwei Purification process of total flavonoids in Kuding tea and study on its xanthine oxidase inhibitory activity Tan Food Research and Development, 2022 (09): 45-48
[10] Shu Xiang, Cheng Ming, Wang Hui, etc Immune regulation and antioxidant effects of Kudingcha Ilex polysaccharides on mouse splenic lymphocytes Zhu Kexue Food Industry Technology, 2022 (17): 23-27
[11] Zhang Xianzhu, Wang Xiaoxiao, Chen Guijie, etc Research progress on polysaccharides in large leaved Kudong tea Chinese Tea Processing, 2022 (01): 12-14
[12] Yin Guoli, Zhao Lu, Zou Chengmei, etc Research on Ultrasonic Assisted Extraction

of Kudong Tea Polyphenols and Their Antioxidant and Hypoglycemic Activities Food Research and Development, 2020 (17): 22-24

[13]Compiled by the Local History Compilation Committee of Luohe City, Luohe City Chronicles, Local Chronicles Publishing House, 1999.03, pages 91-92

[14]Liu Dechao, Jiang Ruirong, Chen Xiaochou, etc Research on the fast propagation technology of white fruit and peach cuttings Shelter Forest Technology, 2017 (8): 30-34

[15]Chen Xiaochou Experimental study on propagation of purple flowered laughing cuttings Fujian Forestry Science and Technology, 2014, 41 (3): 128-131

[16]Wu Chaoxi, Xiao Hui, He Zhibin, etc The effects of plant growth regulators and substrates on the rooting of cherry blossom cuttings Fujian Forestry Science and Technology, 2020, 47 (1): 56-62

