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Study on the Efficient Cutting Propagation Technology for *Ilex* "China Girl"

Xiaobin Wang[1], Yushu Lv[1], Shutang Xing[2], Gang Liu[3], Jingkai Sun[1], Yaqiong Wang[1], Cairu Wang[1], Xiaoyan Yu[1*]

[1]Forestry College of Shandong Agricultural University, Tai'an, China
[2]College of Horticulture Science and Engineering, Shandong Agricultural University, Tai'an, China
[3]Shandong Hanlinyuan Ecological Technology Co. Ltd., Weihai, China
Email: *yxyxst20040214@163.com

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Abstract

In order to realize the efficient industrial production of *Ilex* "China Girl", an orthogonal experiment with 4 factors and 3 levels was designed. Firstly, the optimal orthogonal cutting scheme was selected from 9 treatments. Then through the systematic analysis of the effects of cutting position, substrate, exogenous hormones type and concentration on rooting indexes, such as rooting rate, root number, root length and root effect index, the theoretical optimal scheme was predicted and verified. The results showed that the theoretical optimal scheme (3000 mg/L IBA treatment for 15 s, cutting in mixed matrix with peat soil, perlite and vermiculite ratio of 2:1:1) was the optimal cutting rooting scheme of *Ilex* "China Girl". After the treatment of this scheme, the rooting rate of *Ilex* "China Girl" reached 100%, the average root number was 51.67 per plant, and the average root length was 6.13 cm. The rooting time was greatly shortened, the rooting rate and rooting effect were greatly improved. In this study, the efficient cutting propagation technology system of *Ilex* "China Girl" was established, which laid a foundation for the popularization and application of *Ilex* "China Girl", and also provided reference for further improving the cutting propagation efficiency of other evergreen holly. This study laid a foundation for the application of *Ilex* "China Girl", and also provided a reference for further improving the cutting propagation efficiency of other evergreen holly.

Keywords

Evergreen Holly, *Ilex* "China Girl", Cutting Propagation, Rooting Index

1. Introduction

Evergreen holly has a broad landscape application prospect because of its high

ornamental value, such as evergreen leaves, beautiful tree shape, bright fruit that does not fall in winter [1]. However, the cold resistance of most evergreen holly is poor, cultivated areas in China are mainly concentrated in Jiangsu Province and the south of Jiangsu Province [2] [3] [4] [5]. A few evergreens holly such as *Ilex cornuta*, *Ilex cornuta* "fortunei" and *Ilex crenata* "convexa" can be used in gardens in Shandong Province and surrounding areas. However, their cold resistance is average, and they all suffered severe freezing damage in the extreme low temperature of −18˚C in winter of Shandong Province in 2015.

Ilex "China Girl" is an evergreen holly with strong cold resistance selected by our research group after years of adaptation studies. It is a hybrid of *Ilex rugosa* and *Ilex cornuta*. It is an evergreen shrub with beautiful leaf shape, bright green leaf color, beautiful fruit, full plant type and rapid growth. It can tolerate extreme low temperature of −18˚C in Shandong Province without freezing injury, and is especially suitable for application in Shandong Province and surrounding areas. Therefore, it is of great significance to establish an efficient cutting propagation technology system for *Ilex* "China Girl" to produce a large number of high-quality seedlings as soon as possible and apply them to improve the current situation of winter landscape depression in Shandong Province and surrounding areas.

The cutting propagation techniques of more than 40 evergreen holly species were systematically studied. It was found that the cutting difficulty of different holly species varied greatly, and the survival rate was 20% - 100% [6] [7] [8] [9]. At present, only Tian Weili *et al.* conducted a preliminary study on the cutting survival rate of *Ilex* "China Girl". After 60 days, the rooting rate was 61.75% - 82.75%, and the average rooting number was 5.63 - 9.33 per plant [6]. However, in order to realize the efficient industrial production of *Ilex* "China Girl" and produce a large number of high-quality seedlings rapidly, it is necessary to further shorten the rooting time of cutting and improve the rooting rate and root number of cuttings.

Therefore, *Ilex* "China Girl" was taken as the research object, and the effects of cutting position, substrate, exogenous hormone type and concentration on rooting indexes were systematically studied. The efficient cutting propagation technology system of *Ilex* "China Girl" was established, which laid the foundation for the application of *Ilex* "China Girl", and also provided reference for further improving the cutting propagation efficiency of other evergreen holly.

2. Materials and Methods

2.1. Materials

The materials were *Ilex* "China Girl" introduced from Nanjing in April 2019, and planted in the seedling base of Fanzhen, Daiyue District, Tai'an City, Shandong Province.

2.2. Methods

2.2.1. Materials Drawn

In early September 2020 and early June 2021, the current-year-old branches of

Ilex "China Girl" with strong growth, no pests and diseases, consistent length and diameter were collected.

2.2.2. Cutting Treatment

Each branch was cut into 9 cuttings, with 3 cuttings in each upper, middle and lower part. Each cutting contained 3 axillary buds and retained 2 upper leaves. Cut horizontally at 0.3 cm above axillary bud and obliquely at lower incision. The cuttings were immersed in 1000 times of 80% carbendazim WP for 15 min. Align the biological lower ends of sterilized cuttings downwards, each 60 into a bundle. The 2 cm base of the bundled cuttings was dipped in hormone solutions of different types and concentrations for 15 s (Table 1).

2.2.3. Matrix Preparation

The perlite, peat soil and vermiculite were prepared according to Table 1 and placed in the cutting bed. The day before cutting, sprayed water with a sprayer. On the day of cutting, 80% carbendazim 800 times liquid was sprayed by sprayer.

2.2.4. Cutting

The biological lower end of the cuttings treated according to Table 2 was obliquely inserted into the matrix. Cutting depth was 2 - 3 cm. Leaves didn't contact

Table 1. Test factor level table.

Number	Factors			
	A-hormone type	B-hormone concentration (mg/L)	C-cutting substrate	D-cutting position
1	NAA	1000	perlite	upper
2	IBA	2000	Peat soil: perlite (1:1)	middle
3	NAA:IBA (2:3)	3000	Peat soil: perlite: vermiculite (2:1:1)	lower

Table 2. Cutting treatment scheme.

Treatment	Factors				Combination of factors
	A	B	C	D	
T_1	1	1	1	1	$A_1B_1C_1D_1$
T_2	1	2	2	2	$A_1B_2C_2D_2$
T_3	1	3	3	3	$A_1B_3C_3D_3$
T_4	2	1	2	3	$A_2B_1C_2D_3$
T_5	2	2	3	1	$A_2B_2C_3D_1$
T_6	2	3	1	2	$A_2B_3C_1D_2$
T_7	3	1	3	2	$A_3B_1C_3D_2$
T_8	3	2	1	3	$A_3B_2C_1D_3$
T_9	3	3	2	1	$A_3B_3C_2D_1$
T_L	2	3	3	3	$A_2B_3C_3D_3$

each other. After cutting, the matrix around the cutting hole was compacted, so that the cutting and matrix were closely contacted. Each treatment had 60 cuttings, and the experiment was repeated three times. It should be noted that 9 treatments (T_1 - T_9) were carried out in September 2020, and 2 treatments (T_3 and T_L) were carried out in June 2021.

2.2.5. Post-Cutting Management

After cutting, 1000 times of carbendazim was sprayed once. After that, 1000 times of carbendazim was sprayed once a week for three times. Automatic timing spray equipment was used to adjust the temperature and air humidity during cutting, so that the temperature in the cutting shed was maintained at 20˚C - 38˚C, and the humidity was maintained at more than 80%.

2.2.6. Rooting Index Observation

The base of cuttings treated by different schemes was observed every 10 days after cutting. The rooting rate, root number, root length and root effect index (average root length × average root number/number of cuttings) of 30 cuttings randomly selected were counted at 50 d and 60 d after cutting. It should be noted that 9 treatments (T_1 - $T_{9)}$ in September 2020 were only counted once at 60 d after cutting, and 2 treatments (T_3 and T_L) in June 2021 were counted once at 50 d and 60 d after cutting.

2.2.7. Data Statistical Analysis

Data statistical analyses were performed using SPSS 26 software.

3. Results and Analysis

3.1. Selection of Optimal Orthogonal Scheme

As shown in Table 3 and Figure 1, the rooting rates of T_3, T_5, T_7 and T_9 treatments were 100%. The average root number of T_3 treatment was the highest (49.33 ± 6.17/plant), and the root effect index was the highest (16.20 ± 2.97). Although the average root length of T_3 treatment was only 4.95 ± 0.36 cm, the

Table 3. Rooting indexes of *Ilex* "China Girl" under 9 treatments (September 2020).

No.	Treatment	Rooting rate/%	Average number of roots	Average length of roots/cm	Root effect index
T_1	$A_1B_1C_1D_1$	51.11 ± 3.85D	6.00 ± 1.58D	2.27 ± 0.71C	0.95 ± 0.12E
T_2	$A_1B_2C_2D_2$	98.33 ± 2.89A	45.67 ± 5.51A	4.52 ± 1.31AB	14.07 ± 2.58AB
T_3	$A_1B_3C_3D_3$	100.00 ± 0.00A	49.33 ± 6.17A	4.95 ± 0.36AB	16.20 ± 2.97A
T_4	$A_2B_1C_2D_3$	90.00 ± 3.33B	19.00 ± 3.61C	5.83 ± 0.76A	7.33 ± 1.34C
T_5	$A_2B_2C_3D_1$	100.00 ± 0.00A	22.33 ± 4.62B	4.17 ± 2.31AB	6.50 ± 1.37C
T_6	$A_2B_3C_1D_2$	84.45 ± 3.85B	23.67 ± 1.59B	3.83 ± 0.29B	6.11 ± 1.25C
T_7	$A_3B_1C_3D_2$	100.00 ± 0.00A	29.67 ± 4.29B	5.90 ± 0.57A	11.67 ± 1.89B
T_8	$A_3B_2C_1D_3$	73.33 ± 6.67C	16.67 ± 1.21C	2.63 ± 0.98C	3.37 ± 0.50D
T_9	$A_3B_3C_2D_1$	100.00 ± 0.00A	19.33 ± 3.06C	5.50 ± 1.32A	7.09 ± 2.18C

Figure 1. Rooting state of *Ilex* "China Girl" under 9 treatments (September 2020).

difference was not significant compared with that of T_7 treatment (5.90 ± 0.57 cm/plant) with the longest average root length. Therefore, T_3 treatment (A1B3-C3D3), *i.e.*, the lower cuttings were dipped in 3000 mg/L NAA solution for 15 s, and cut in mixed matrix with peat soil, perlite and vermiculite ratio of 2:1:1, was the optimal solution in 9 treatments (T_1 - T_9).

3.2. Prediction of Optimal Theoretical Scheme

3.2.1. Effects of Hormone Type on Rooting Indexes

As shown in Table 4, the rooting rate of cuttings treated with IBA alone was the highest and significantly higher than that treated with NAA alone. The average number of roots treated with NAA alone was the highest and significantly higher than that treated with IBA alone and NAA: IBA (2:3). Many researchers generally believed that rooting rate was the most important in four rooting indexes. So IBA was the best rooting hormone.

3.2.2. Effects of Hormone Concentration on Rooting Indexes

As shown in Table 4, the rooting rate and average root number of cuttings treated with 3000 mg/L hormone were the highest and significantly higher than those treated with 1000 mg/L hormone. Therefore, the optimal rooting hormone concentration was 3000 mg/L.

3.2.3. Effects of Substrate Type on Rooting Indexes

As shown in Table 4, all rooting indexes of peat soil: perlite: vermiculite (2:1:1) were the highest, and 4 indexes were significantly higher than perlite, 2 indexes were significantly higher than peat soil: perlite (1:1). Therefore, the optimal cutting substrate was peat soil: perlite: vermiculite (2:1:1).

In addition, by comparing the range values of different rooting indexes under different influencing factors, it was found that the matrix type had the greatest impact on all rooting indexes (R = 30.37), followed by hormone concentration (R = 14.45), and then the cutting position (R = 10.56) and hormone type (R = 8.34). Therefore, matrix type was the most important factor affecting rooting

Table 4. Multiple comparison and extreme difference analysis of orthogonal experiment (September 2020).

Factors	Levels	Rooting rate/%	Average number of root	Average length of root/cm	Root effect index
A-hormone type	NAA	83.15b	33.67a	-	-
	IBA	91.48a	21.89b	-	-
	NAA:IBA(2:3)	91.11a	21.67b	-	-
	R	8.34	12.00	1.10	3.76
B-hormone concentration	1000	80.37b	18.22b	-	-
	2000	90.56a	28.22a	-	-
	3000	94.82a	30.78a	-	-
	R	14.45	12.55	1.23	2.47
C-substrate	perlite	69.63b	15.44c	2.91b	3.48c
	Peat soil:perlite (1:1)	96.11a	28.00b	5.28a	9.50b
	Peat soil:perlite:vermiculite (2:1:1)	100.00a	33.78a	5.34a	12.14a
	R	30.37	18.33	2.43	8.66
D-cutting position	upper	83.70c	15.89b	-	4.85b
	middle	94.26a	28.33a	-	11.30a
	lower	87.78b	33.00a	-	8.97a
	R	10.56	17.12	1.10	6.45

index, followed by hormone concentration.

In short, according to the results of multiple comparison and range analysis of orthogonal experiment, $A_2B_3C_3D_2$, that was, using 3000 mg/L IBA to treat middle cuttings for 15 s, cutting in peat soil: perlite: vermiculite (2:1:1) mixed matrix, was the best theoretical scheme for cutting rooting.

3.3. Verification of Optimal Theoretical Scheme

From June to August 2021, the rooting indexes of the optimal theoretical scheme T_L and the optimal orthogonal scheme T_3 were observed regularly. It was found that all the rooting indexes of T_L treatment were higher than those of T_3, and the rooting indexes of 50 d after treatment were higher than those of 60 d after treatment (Table 5). Therefore, T_L treatment ($A_2B_3C_3D_2$) was the best cutting rooting scheme.

4. Discussion

There are many factors affecting plant cutting propagation, including internal factors such as plant species (varieties), cutting age, sampling site, cutting specification, and external factors such as matrix, temperature, humidity, light, plant growth regulators [10] [11] [12] [13]. The results showed that substrate type was

Table 5. Rooting indexes of T_3 treatment and T_L treatment (June 2021).

Treatment	Combination of factors	Cutting time	Rooting rate/%	Average number of root	Average length of root/cm	Root effect index
T_3	$A_1B_3C_3D_3$	50d	100.00 ± 0.00A	40.63 ± 5.28B	4.05 ± 0.22C	10.89 ± 1.65D
		60d	100.00 ± 0.00A	48.13 ± 5.43AB	5.25 ± 0.26B	16.09 ± 2.11C
T_L	$A_2B_3C_3D_2$	50d	100.00 ± 0.00A	51.67 ± 5.48AB	6.13 ± 0.34AB	20.98 ± 2.53B
		60d	100.00 ± 0.00A	59.48 ± 6.03A	7.26 ± 0.58A	28.56 ± 3.72A

the most important factor affecting rooting index of *Ilex* "China Girl", followed by hormone concentration.

Ideal cutting substrate should have good air permeability, moderate water retention and drainage, no bacterial infection, and certain fertility. However, different plants have different requirements for water retention and drainage [14] [15]. In this study, four rooting indexes in the mixed matrix containing 50% peat soil were significantly higher than those in perlite for *Ilex* "China Girl". This may be because *Ilex* "China Girl" requires relatively high substrate humidity, but the water retention of perlite cannot meet its requirements.

Exogenous hormone treatment can significantly improve the rooting rate and rooting effect of cutting, but the optimal hormone types and concentrations of different plants are different [16] [17]. In this study, it was found that IBA was the optimal rooting hormone for *Ilex* "China Girl". When other treatments were the same as those in this study, the highest rooting rate of *Ilex* "China Girl" treated with ABT by Tian *et al.* was only 61.75%, and the average root number was only 8.61/plant [6]. In this study, the rooting rate was up to 100% and the number of roots was up to 45.67/plant after IBA treatment, which greatly improved the rooting rate and rooting effect of *Ilex* "China Girl". In addition, this study also found that the effect of 2000 - 3000 mg/L hormone treatment was significantly better than 1000 mg/L hormone treatment, and with the increase of hormone concentration, the rooting rate and average root number were further improved. However, whether further increasing hormone concentration can further improve rooting rate and average root number still needs experimental verification.

5. Conclusion

In this study, the optimal cutting rooting scheme of "Chinese young girl" was developed, that is, using 3000 mg/L IBA to treat middle cuttings for 15 s, cutting in peat soil:perlite:vermiculite (2:1:1) mixed matrix. After the treatment, the cutting rooting time of *Ilex* "China Girl" was greatly shortened, and the rooting rate and rooting effect were greatly improved.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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