Optimizing the Propagation of *Cinnamomum micranthum* by Cuttings

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Abstract: *Cinnamomum micranthum* (Hayata) Hayata is a rare but economically important tree species native to Taiwan and mainland China with poor natural regenerative capacity and recalcitrance to propagation via seed. Clonal propagation methodology was revised aiming to optimize the level of plant growth regulator used and the cutting type. Out of the range 100 – 2000 ppm of IBA tested, the level of 500 ppm was optimal with a rate of 70.6% of cuttings rooted. The cutting type (tip mid or basal section of branch) also significantly affected the rate of rooting with the tip cutting providing the highest rooting rate (73.2%) with significantly and progressively lower rooting obtained on more basal sections. In order to maximize rare scion resources of *C. micranthum*, it is recommended that in addition to tip cuttings, the midpiece of branch should be taken as the cutting scion as it was possible to obtain satisfactory rates (50.1%) of rooting on this cutting type as well.

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1. Introduction

Cinnamomum micranthum (Hayata) Hayata is a rare but economically important tree species native to Taiwan and mainland China. In China it is found at the foothills of mountains up to an altitude of 600 meters in Guangxi, Guangdong, provenances, and the north to Nangjing and Anxi in the Fujian Province. There has been a long history of destruction of *C. micranthum* leading to its rarity, with the only large distribution of this species in Fujian now limited the Jian'ou Wanmulin Reserve, Fujian Province, China. This population is gradually dying leading to the listed of this species on the National Third Levels of Endangered Species in China. *C. micranthum* produces an excellent quality timber for ship- and furniture-making as it possesses the qualities of a pleasing fragrance, straight wood texture, and a uniform and exquisite structure. The trees are eligible for extracting aromatic oil which contains the important industrial material-safrole. It is also a popular ever-green ornamental, with a tall, straight trunk, and attractive glabrous broad leaves (He, 2013).

Several features of the reproductive biology of *C. micranthum* lead to low levels of regeneration and difficulty of propagation by seed. It is a self-pollination tree species, and the male and female floral development times are different causing high the empty fruits rate. It takes 15 years to mature and produce viable offspring. If mass fruiting happens before the tree is mature, the percentage of the vacant fruits reaches 75%, and much fruit is lost due to herbivory. The seeds contain abundant oil, have short lifetime, thus are difficult to store and maintain high germination rates. The seedlings of *C. micranthum* are difficult to collect. Hence there is a strong need to develop clonal propagation techniques for *C. micranthum* to provide material for afforestation.

The cutting propagation of *C. micranthum* was studied (Zhang, 2000; Geng, 2006; Feng *et al*., 2007), and the highest rooting rate was 51.7% (Zhang, 2000) or 50％ (Feng *et al*., 2007) and as yet these approaches have been unable to provide sufficient clonal material for afforestation. Therefore, we revisit cutting propagation for this species with the aim of further optimizing propagation parameters to support afforestation of this important economic tree species.

2. Material and Methods

2.1 The Test Site

The test site is located at the nursery of Fujian Academy of Forestry Sciences, China. It belongs to the subtropical maritime monsoon climate zone, and has mild climate and plentiful rainfall. The frost free period lasts for 326 days per year. Annual average temperature is 19.6 C, annual humidity 77%, and annual rainfall 1342.5 mm. The average temperature during the coldest January is 10.5 C, and the average temperature during the hottest July is 28.6 C.

2.2 Media preparation

The local new red-core soil was pulverized to form the media for cutting propagation. Plastic basins with drain holes, length 40cm×width 30cm×height 30cm were filled with the soil then placed in a greenhouse with 70% shade level. The media was then sterilized by 0.1‰ $KMnO_4$ and covered by the plastic film for 3 days prior to use.

2.3 The source of the scions

In September 2013 scions were taken from 10-year-old stock trees of *C. micranthum* in Jian'ou City. The stock trees were cut at the height of 1 meter 1 year earlier to induce juvenile shoots suitable for scions. Pests and disease free, vigorously growing branches were selected for scion material.

2.4 Experimental design

Two factors were investigated for optimization, the level of the plant growth regulator, indole-3-butyric acid (IBA), and cutting type (tip, mid or basal section of branch). Due to the low amount of scion material because of the rarity of the stock plants, factors were optimized sequentially. In the first experiment, IBA concentration of (1) 100 ppm, (2) 500 ppm, (3) 1000 ppm, (4) 2000 ppm, were tested. The scions were taken from Jian'ou Fujian to Fuzhou Fujian within 6 hours. A randomized block design was used in this experiment, with 200 scions for each treatment and 3 replicates. The root system was rinsed and the cleaned roots were assessed to derive rooting rate, root number per individual cutting, and root length parameters. In the second experiment, cutting type was investigated utilizing the optimal rate of IBA as determined in the first experiment. The cutting type experiment utilized a randomized complete block design with 300 cuttings of each cutting type and 3 replicates.

2.5 Scion treatment

The 8-15cm length branches with 3-5 leaves were taken as the scions. The top leaves were kept but lower leaves were cut half (See Figure 1). After cutting the scions were immersed into the water in a large basin to prevent dehydration. Then all the scions were taken out and immersed into the solution of 1000 fold carbendazim (effective constituent 50%) for 10-15 minutes. The bases of scions were dipped with the different rooting reagent for 5 minutes, then put into the soil to a depth of 2/5-1/2 scion length by using a bamboo stick digging a hole prior to insertion of the scion. 30 scions were put in each plastic basin. The soil was pressed tightly after the scions were inserted then the soil was fully watered.

2.6 Scion management

To maintain high humidity the tubs containing the scions were enclosed in a plastic tent. The soil and atmosphere was keep moist with an automated misting system by a supplemented with hand-watering with the frequency of once a day on sunny day or once per 2-3 days on cloudy or rainy day. The plastic tent was removed for hand watering but immediately replaced after watering. The scions were sprayed on the solution of 800 fold carbendazim (effective constituent 50%) or mancozeb (effective constituent 80%) once a week. After 6 months (i.e. March of the following year) most of the buds emerged, and the film was gradually taken off. The mist frequency was increased and the soil was kept wet. Two weeks after the film was taken off, the survival rate, rooting number per individual and root length were determined.

2.7 Statistics analysis method

The data were analysed using SPSS Statistics 17.0 analysis of variance (ANOVA) and a post-hoc Tukey's test if the ANOVA was significant. Means are provided with standard errors, and means considered significantly different at $P<0.05$.

3. Results

3.1 The effect of IBA concentration on the rooting of *C. micranthum*

The experiment to test the effect of IBA concentration upon rooting of *C. micranthum* cuttings was established on Sep 26th, 2013. Rooting was assessed after six months on March 18th, 2014. The three aspects of rooting evaluated, rate, average root number per individual, and average root length exhibited homogeneity of variance (data not shown). ANOVA indicated that there were significant differences in rooting rate among the 4 IBA treatments (Table 1). The 500ppm treatment produced a rooting rate of 70.6% which was significantly higher than all other treatments. The 100 and 1000ppm treatments produced lower rooting rates but were significantly higher than the highest IBA level of 2000ppm. Significant differences were also found for the average root number per individual, with the highest level obtained in 1000ppm treatment, followed by the 100ppm and 500ppm treatments, and the lowest rate with 2000ppm treatment. Significant differences were also found for average root length, with the highest to the lowest. From the best to the worse obtained from treatments in the following order: 100 > 1000 > 500 > 2000.

The number of cuttings which root and the quality of the root systems are both important parameters for optimization of propagation protocols. The root systems on treatments from 100 to 1000ppm were all adequate based on root length and average root number, however, as 500 ppm rate achieved a significantly higher rate of rooted cuttings, it is the recommended level for use with *C. micranthum*.

3.2 The effect of cutting type upon rooting; basal verses tip cuttings

Using the optimized IBA level (i.e.500 ppm IBA) determined above, we next tested whether the type of cutting (tip, mid or basal) affected rooting rates. The experiment was established on May 24th 2014 with rooting rates assessed on July 27th 2014. A one-way ANOVA showed that there was a significant difference in rooting rate due to cutting type and that the rooting rate exhibited homogeneity of variance (data not shown). The highest level of rooting was obtained from the tip of the scion (73.2%), with significantly and progressively lower levels from the mid and basal sections (Table 2 and Figure 1).

Table 1. The effect of IBA concentration upon rooting in *C. micranthum*

Test No.	IBA concentration /ppm	Rooting rate /%	Average root number per individual	Average root length /cm	Note
1	100	45.0±1.74 b	10.4±0.64 b	6.05±0.51 a	Plenty of fiberous roots
2	500	70.6±2.94 a	10.5±0.70 b	4.20±0.21 bc	Plenty of fiberous roots
3	1000	47.1±2.16 b	15.1±1.16 a	5.23±0.20 ab	Plenty of fiberous roots
4	2000	25.6±1.35 c	9.2±0.70 b	2.92±0.09 c	Less fiberous roots

NB：Significant differences among treatments tested by Tukey’s test; treatments with post-scripted with different letters were significantly different at the 95% level.

Table 2. Rooting rates for cutting type

Test No.	Different part of branches	Average rooting percentage /%	Note
1	Tip	73.20±0.78 a	Apical buds grew
2	Mid	50.10±2.43 b	Axillary buds grew instead of apical buds
3	Base	12.82±1.57 c	Axillary buds grew instead of apical buds

Figure 1. A tip cutting with roots (left) and Cutting seedlings of *C. micranthum* (right)

4. Discussions

4.1 In general, the optimal plant growth regulator and its concentration for the cutting propagation of *C. micranthum* was 500 ppm IBA with which the rooting rate could reach 70.6%. The branch with apical bud was suitable to be the cutting scion, with which the rooting rate could reach 73.2%. IBA was adopted to be the rooting reagent in this experiment, which was different from the reagent H_2O_2 used by Zhang (2000) and ABT used by Feng *et al* (2007). The rooting rate in this experiment was 70.6-73.2% which was higher than 50％-51.7% that was obtained by Zhang (2000) and Feng *et al* (2007).

4.2 Some researchers in China studied the cutting propagation of *Cinnamomum camphora* which is the same family and same genus to *C. micranthum*. Liu *et al* (2003) used 400 ppm ABT1 (Produced by Chinese Academy of Forestry) with which the rooting rate achieved 79.3%-94.3%；Huang *et al* (2008) used 100 ppm ABT treating for 720 minutes or 500 ppm ABT treating for 10 minutes with which the rooting rate achieved was 85%. Zhang *et al* (2006) used 300-500 ppm ABT with which the rooting rate achieved was 90-100%. Long *et al* (1990) used 50 ppm IBA or NAA treating for 23 hours with which the rooting rate achieved was 76-84%. The former, Liu *et al* (2003), Huang *et al* (2008), and Zhang *et al* (2006) , adopted ABT but the latter, Long *et al* (1990) , used IBA, which was the same plant growth regulator as was used in this experiment, but all the cutting rooting rates reported for *C. camphora* were higher than that of *C. micranthum* in the current experiment. The higher rates of rooting obtained for *C. camphora* is believed to be due to the fact that the branch of *C. micranthum* has medulla which is easy infected by fungus or bacteria from the soil, hence more cuttings are lost due to disease.

4.3 Xiang (2014) reported the effect of different parts of the branch on the rooting rate of *C. micranthum*. The result showed the top part with apical buds was the best one for cutting scions whether it was lignification or non-lignification branches, which is the same result to this experiment. Endogeneous hormone levels often differ along the branch. Also there is an ontogenetic gradient with more physiologically mature tissues towards the base of the branch.

4.4 Because stock trees of *C. micranthum* are very rare, in order maximize available materials for propagation, it is recommended that although the rooting rate for the mid-part of the branch was only 50.1%, this section of the branch should also be utilized until stock plant resources are increased.

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References

1. Feng LZ, Chen YZ, Ma XQ, Huang YL, Zheng QR). Cuttage seedlings of *Cinnamomun micranthum*, an endangered plant. Journal of Fujian College of Forestry 2007; 4: 333-336.
2. Geng YM. The Experiment of Cutting Propagation of *Cinnamomum micranthum*. Journal of Fujian Forestry Science and Tehcnology 2006; 3: 151-154.
3. He GS. Coloured Illustrations of the Trees in Fujian Province. Xianmen University Publisher, Xiamen China. 2013: 97.
4. Huang JS, Wei YW, Chen XM, Wu YM, Yu HQ. The cutting propagation technique of the juvenile branches of *Cinnamomum camphora* var. *1inaloolifera*. Guangxi Tropic Agriculture 2008; 5:1-3.
5. Liu DL, Jin JL. Study on Cutting Propagation of *Cinnamomum camphora*. Journal of Fujian College of Forestry 2003; 2:189-192.
6. Long GY, Liu YJ, Guo DX. The Preliminary Report on the Cutting Propagation Test of *Cinnamomum camphora*. Jiangxi Forestry Science and Tehcnology 1990; 1: 1-6.
7. Zhang JZ, Yao XH, Ren HD, Wang KL, Li S, Xu YQ. Study on Cutting Propagation of *Cinnamomum camphora* Presi. Forest Research 2006; 5: 665-668.
8. Xiang F. Influence of the Location of Cutting Slips on Cutting Rooting of *Cinnamomum camphora* (L.) Presl. Journal of Sichuan Forestry Science and Technology 2014; 1:63-64.
9. Zhang SY. A Preliminary Study on Cutting Propagation Techniques for Old Plants of *Cinnamomum micranthum*. Journal of Fujian Forestry Science and Technology 2000; 4:69-71.

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