Research paper

Cutting Propagation by Water Culture of *Casuarina equisetifolia*

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【Summary】

Asexual reproduction ensures that advantageous genetic traits and characteristics of parental trees are passed onto progeny. Furthermore, seedlings grow very fast during the developmental period. To enhance the population size and improve reproduction of *Casuarina equisetifolia*, this study investigated how various factors influence the rooting quality of water-cultured *C. equisetifolia*. Using a recently developed technique, young branch cuttings were grown in water culture. To identify optimal conditions for water culture reproduction, various cuttings aged 3 and 6 mo were expored to a napthaleneacetic acid (NAA) concentration of 50 ppm. Cutting lengths of < 10 cm had better rooting quantity than 10~15-cm cuttings. The rooting percentage of cuttings with wound treatment was 86.7% compared to 73.3% for cuttings without wound treatment. The cutting types used in this 'water culture' of rooting quantity were 2.1 for cuttings with branches and 1.8 for those without branches. The results of this study can be used to develop techniques to improve the rooting rate and lower the costs of growing *C. equisetifolia* seedlings for use in coastal regions of Taiwan.

Key words: *Casuarina equisetifolia*, water culture, rooting quality.

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研究報告

木賊葉木麻黃之水培繁殖

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摘 要

無性繁殖可保留母樹的遺傳特性和特徵，且幼苗期之生長更為迅速。本研究使用近來發展的木麻黃嫩枝插穗於水中培植發育之技術，分析各種影響發根品質之因素，以了解處理差異與提升發根品質，研究透過3~6個月內之木麻黃嫩枝插穗，應用萘乙酸濃度50 ppm之發根劑濃度有較佳發根率(82.2%)。木麻黃嫩枝插穗長度10公分以內發根品質最好，木麻黃嫩枝之插穗切基處理有較佳發根率(86.7%)，木麻黃嫩枝插穗之分叉具發根2.1多於未分叉1.8之發根數，等結果有助木麻黃水培繁殖之發根品質改善；此結果可發展為高發根率且低成本之育苗技術，提供台灣海岸更新之選擇。

關鍵詞：木麻黃、水培、發根品質。

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INTRODUCTION

Casuarina equisetifolia is a subtropical tree commonly found in Australia, Southeast Asia, and the Pacific islands (Wilson and Johnson 1989). These tree are most prevalent in China and India where they are used for a number of purposes including providing trees for purely esthetic purposes, wind belts, and sand protection in coastal areas, as well as wood pulp, medicine, tanning, and dyes (Doran and Hall 1983, Pan and Li 1996, Zhong et al. 2005). Since *C. equisetifolia* was imported in 1897, it has been used for wind resistance and sand protection along the coasts of Taiwan for over 100 yr. *Casuarina equisetifolia* is considered one of the pioneer trees along coastal areas of Taiwan. An experiment on the southern coast of China proved that *C. equisetifolia* is the most suitable tree to plant on coastal sand land in tropical and subtropical areas (Pan and Lu 1990, Huang et al. 2006). However, as diseases and pests can affect vast areas of *C. equisetifolia* plantations and naturally occurring populations, their average life is approximately 20~30 yr in local coastal areas. In addition to this shortened lifespan and problems associated with reproduction, continuous afforestation is required.

While sexual reproduction occurs in *C. equisetifolia*, the observed lack of variation (He et al. 2005) limits the use of such seeds. Initially, China lacked good-quality seed resources to resist the stress of extreme environments. Consequently, an initiative to find a quick method of asexually generating disease-resistant *C. equisetifolia* using water culture was established. When *C. equisetifolia* reproduces asexually, advantageous genetic traits and characteristics of parental trees are inherited by all progeny, thereby increasing the production of large quantities of good-quality seedlings (Ke et al. 2001, Chen et al. 2003).

Water culture is the primary asexual reproduction technique used for studying *C. equisetifolia* in China (Lou 2002, Lin 2007). This technique involves taking cuttings and

immersing them in water. A study of *C. junghuhniana* demonstrated that the rooting quality could be improved by altering the age of the cuttings, the concentration of (indole-3-butric acid, IBA), strengthening cutting work in scion production orchards, and cutting branches to grow new offshoots to renew parental trees (Chen et al. 1995). As nursery forests in coastal areas of Taiwan are steadily deteriorating, it is very important to renew *Casuarina* forests. Seedling quality and adaptability are therefore extremely important issues. Experiments in China led to the selection of good-quality seedlings that successfully been grown in coastal forests via water culture. Using these techniques, we atlempted to increase the growth, disease tolerance, and wind resistance of trees to renew coastal *Casuarina* forests in Taiwan in the future.

MATERIALS AND METHODS

Establishment of a scion production orchard of *C. equisetifolia* seedlings

The experimental materials were obtained from Fangyuan in Changhua on the west coast of Taiwan. The seeds were taken from well-formed *C. equisetifolia* from Wuqi wind break forest in Taichung. Seedlings were cultivated from seeds that we obtained. Seedlings were 1 yr old and had an average height of 100 cm. The average stem base diameter was 1.5 cm. Seedlings were subsequently moved to the nursery of the Department of Forestry and Natural Resources, National Chiayi University to establish a scion production orchard. After the seedlings began to grow, a branch was cut at a point 30~40 cm from the ground, and offshoots and soft young cuttings were taken as experimental materials. Other cuttings of uncut plants were taken as an experimental comparison group used in subsequent experiments.

Water culture experiments

The experimental conditions used in the water culture experiments included variations in cutting age and concentration of napthaleneacetic acid (NAA), comparison of rooting quality of different species, and measurement of cutting lengths, the rooting quality of different wounding treatments, and rooting quality of different types of cuttings. All experiments were performed during June and July 2007.

Effects of cutting age on rooting quality

Cutting materials included a range of offshoots aged 3, 6, and 24 mo. To make cuttings to specifications which could be standardized, cuttings with a length of 10~12 cm were selected. Before insertion, the bottoms of the cuttings were removed with scissors, producing cuttings with a length of 8~10 cm. Then the bottom 2~3 cm of the cutting was immersed in 50 ppm NAA for 24 h. After 24 h, the immersed cuttings were rinsed in clean water and re-immersed in 3~4 cm of water and placed in the sun. The container used in the immersion operation was a 2-cm-diameter experimental glass tube with height of 6.5 cm. Water in the container was changed daily.

Three ages of cuttings were compared in the experiment, and cuttings were subsequently washed with clean water before immersion. The experimental area was divided into 6 blocks, each containing 15 cuttings. The total number of cuttings in the experiment was thus 15 (number of cuttings)$\times$3 (age of the cutting)$\times$6 (number of blocks) = 270 (cuttings). The 1st rooting date was recorded. We counted the quantity and root growth of the cuttings. The rooting percentage was determined according to the number of living cuttings, and the dead percentage was determined based on the number of dead cuttings. Angle transformation was performed

before 2 data analyses of the latter. Analysis of all data was performed using SPSS 12.0 software (IBM acquires SPSS Ins., Chicago, IL, USA).

Effects of NAA concentration on rooting quality

This experiment examined the effects of NAA on promoting root quality. The experimental materials were comprised of offshoots from cut branches of the parental plant. Cuttings were taken at 0.5~1 cm from the base. Then, the cuttings were immersed in 25, 50, 75, and 100 ppm of NAA. Cuttings without NAA (0 ppm) were prepared as a control group. After soaking in a fixed concentration of NAA for 24 h, cuttings were immersed in media. The experimental area was arranged according to blocks. There were 6 blocks, each containing 15 cuttings. The total number of cuttings in the experiment was 15 (number of cuttings) × 5 (concentration) × 6 (number of blocks) = 450 (cuttings).

Effects of cutting length on rooting quality

The experimental materials were the same as those in the above experiment. We used 3 different lengths of *C. equisetifolia* for the experiment: < 10, 10~15, and > 15 cm. All cuttings were taken 0.5~1 cm from the base, soaked in 50 ppm NAA for 24 h, and subsequently washed in clean water before insertion. The experimental area was organized according to blocks. There were 6 blocks, each containing 15 cuttings. The total number of cuttings in the experiment was 15 (number of cuttings) × 3 (length of the cutting) × 6 (number of blocks) = 270 (cuttings).

Effects of wounding treatments on rooting quality

The materials were the same as in the above experiment involving offshoot cuttings from the scion production orchard. The cutting lengths were 8~10 cm. All cuttings were taken 0.5~1 cm from the base, soaked in 50 ppm NAA for 24 h, and washed with clean water before immersion. Non-wound-treated cuttings were prepared for comparison with wound-treated cuttings. The experimental area was divided according to blocks. There were 6 blocks, each containing 15 cuttings. The total number of cuttings in the experiment was 15 (number of cuttings) × 2 (treatment) × 6 (number of blocks) = 80 (cuttings).

Effects of cutting type on rooting quality

The materials were the same as in the above experiment of offshoot cuttings from the scion production orchard. Branched and non-branched cuttings were used in this experiment. Cuttings had lengths of 8~10 cm. All cuttings were taken 0.5~1 cm from the base, soaked in 50 ppm NAA for 24 h, and subsequently washed with clean water before immersion. The experimental area was divided according to blocks. There were 6 blocks, each containing 15 cuttings. The total number of cuttings in the experiment was 15 (number of cuttings) × 2 (types) × 6 (number of blocks) = 180 (cuttings).

RESULTS AND DISCUSSION

Effects of cutting age on rooting quality

After 30 d of rooting, notable differences in the time it took each age of cutting to produce roots were observed (Table 1). Roots appeared on 3-mo cuttings 8 d after transfer compared to 17 d for 2-yr cuttings. Various cuttings also had very different rooting percentages. For example, the 3-mo cuttings had the highest rooting percentage at 86.7% of all plants bearing roots, while the 2-yr cuttings had the lowest rooting percentage at 8.9%. Differences in root length were also apparent.

Table 1. Rooting quality of different-aged cuttings

Rooting quality	Age of cutting (mo)			*F*-value
	3 (n = 90)	6 (n = 90)	24 (n = 90)	
Rooting percentage (%)	86.7±6.3[a,1)]	78.9±5.3[a]	8.9±10.5[b]	75.8**
Death percentage (%)	0.1[b]	0.1[b]	46.7±6.3[a]	944.3**
Length (cm)	1.4[a]	1.3[a]	0.5[b]	7.9**
Rooting number	2.0[a]	1.8[a]	0.5[b]	29.6**

[1)] * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$; Denote significant differences according to Duncan's multiple-range test.

[2)] Different letters indicate a significant difference of the means at the 0.05 level among detection methods by Duncan's new multiple-range comparison, mean±standard error.

[3)] Numbers in parentheses are percentages of the total.

The average length of 3-mo cuttings was 1.4 cm, while the average length of 2-yr cuttings was 0.5 cm. Furthermore, the death rate varied from 46.7% for 2-yr cuttings to 0% for both the 3-and 6-mo cutting populations.

These results show that rooting quality of cuttings aged 3 and 6 mo was much better than that of cuttings aged 2 yr. This result is consistent and indicates that ripe wood has greater difficulty rooting (Ke et al. 2001). In addition, the rooting quality of ripe cuttings was lower than that of young cuttings (Gill 1983). Different types of wood have varying rejuvenating lives, and this was reflected in our data whereby the rooting rate of 2-yr cuttings was reduced to 8.9%, making such cuttings unsuitable for water culture (Morgenstern 1987). However, when seedling branches of 2-yr-old cuttings were cut, 3-mo cuttings of the branches had the highest rooting rate. This means that branch cutting has a rejuvenating effect and can provide a method to research rejuvenating materials (Kao and Huang 1993). Scion production orchards can be established in the future with seed resource selection and nursery.

Effects of NAA concentration on rooting quality

Rooting quality also seemed to be influenced by different concentrations of NAA (Table 2). Cuttings treated with 50 ppm NAA

Table 2. Effects of NAA concentrations on the rooting quality of cuttings

Rooting quality	NAA concentration (ppm)					*F*-value
	0 (n = 90)	25 (n = 90)	50 (n = 90)	75 (n = 90)	100 (n = 90)	
Rooting percentage (%)	0.1[d]	14.4±19.0[c]	90.0±5.9[a]	78.9±5.3[a]	48.9±9.6[b]	68.8**
Death percentage (%)	20.0±12.5[a]	12.2±10.3[a,b]	0.1[c]	0.1[c]	3.3±5.9[b,c]	8.1**
Length (cm)	0.1[d]	0.6[c]	1.6[a]	1.0[b]	1.0[b]	22.2**
Rooting number	0.1[d]	0.4[c]	2.1[a]	1.9[a]	1.3[b]	72.0**

[1)] * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$; Denote significant differences according to Duncan's multiple-range test.

[2)] Different letters indicate a significant difference of the means at the 0.05 level among detection methods by Duncan's new multiple-range comparison, mean±standard error.

[3)] Numbers in parentheses are percentages of the total.

were the first to root with rooting occurring on the 8th day. For cuttings treated with 25 ppm, rooting occurred on the 13th day. Rooting percentages markedly differed among samples. The rooting percentage of cuttings treated with 50 ppm was the highest at 90%, while cuttings treated with 0 ppm had the lowest rooting rate of 0%. Differences in rooting number also greatly differed. Cuttings treated with 50 ppm had the highest average number of rooted cuttings. Meanwhile, the lowest average number of rooted cuttings was for those treated with 25 ppm. Rooting lengths also markedly differed.

The length of the longest average cutting was in the group treated with 50 ppm, while the shortest average length was in the group treated with 25 ppm. The percentage that died also markedly differed. The percentage of cuttings of the control that died was the highest. Furthermore, the lowest death rate of cuttings was among those treated with 50 and 70 ppm NAA.

The experimental results showed that the concentration of NAA significantly improved rooting and viability, with 50 ppm NAA being identified as the optimum concentration for water culture. Previously, Chen et al. (1995) took cuttings of *C. junghuhniana* from a scion production orchard at Pizitou and tested the effect of various concentrations of IBA on rooting. In that study, despite being limited by ripeness, the rooting rate of cuttings not treated with rooting hormone was extremely low, and 3000 ppm IBA promoted the formation of adventitious roots. However, while the rooting rate was high, the rooting number was large, and the rooting quality was enhanced, 4000 ppm IBA is lethal to *C. equisetifolia*. Somasundaram and Jagade (1977) noted that plant hormones can promote the growth of side branches of *C. equisetifolia*. While untreated cuttings did not root at all, the rooting rate of cuttings treated with 50 ppm was the highest. Rooting is very sensitive to hormone concentractions. High concentrations can inhibit root growth, reduce rooting quality, decrease viability (Lindquist and Torrey 1984), and even cause death (Wise et al. 1985, Ponchia and Howard 1988).

Effects of cutting length on rooting quality

Comparing different-length cuttings revealed that cuttings of < 10 cm displayed better rooting quality than 10~15-cm cuttings (Table 3). The rooting rate also differed, with the rooting rate of cuttings of < 10 cm being the highest, while the rooting rate of cuttings of > 15 cm the lowest. Furthermore, the average rooting number of cuttings of < 10 cm

Table 3. Rooting quality of different-length cuttings

Rooting quality	Length of cutting (cm)			*F*-value
	< 10 (n = 90)	10~15 (n = 90)	> 15 (n = 90)	
Rooting percentage (%)	87.7±5.3[a]	41.1±5.3[b]	0.1[c]	774.0**
Death percentage (%)	0.1[b]	21.1±12.1[b]	94.4±9.3[a]	16.2**
Length (cm)	1.4[a]	0.9[b]	0.0[c]	285.8**
Rooting number	2.1[a]	1.4[b]	0.0[c]	196.0**

[1] * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$; Denote significant differences according to Duncan's multiple-range test.

[2] Different letters indicate a significant difference of the means at the 0.05 level among detection methods by Duncan's new multiple-range comparison, mean±standard error.

[3] Numbers in parentheses are percentages of the total.

was the highest. Similarly, the rooting length also considerably varied, with the longest average rooting length of cuttings found for cuttings of < 10 cm, and the shortest length for cuttings of 10~15 cm. Finally, the percentage of cuttings that died also considerably differed, ranging 94.4 to 0% for cuttings of > 15 cm and those < 10 cm. Results of this experiment indicate that the rooting rate decreased with on increase in the cutting length.

Effects of different wounding treatments on rooting quality

Thirty days after wounding treatment and non-wounding treatment, the rooting of cuttings with wounding treatment appeared first on the 8th day. Rooting of cuttings with no wounding treatment appeared on the 12th day. The rooting percentage of cuttings with wounding treatment was higher than that without wounding treatment. Rooting quantity and the length of cuttings also varied (Table 4). Surprisingly, the death rates of the 2 types were the same.

During water culture, tubercle-shaped concrescences of the cuttings with a lower rooting percentage were clearer than the ones with a higher rooting percentage. The concrescences of cuttings without wounding treatment were larger than those of cuttings with wounding treatment in this experiment. Ke (2001) suggested that wounding treatment removed the old concrescences of cuttings, enabling the plant hormones to directly function at the site of the incision.

Effects of different cutting types on rooting quality

Rooting of cuttings with branches appeared first, on the 8th day (Table 5). Meanwhile, rooting of cuttings with no branches appeared on the 10th day. The rooting rate did not differ between the 2 groups, at 87.8% in both cases. However, the rooting quantity considerably differed, being 2.1 for cuttings with branches and 1.8 for those without branches. Finally, the 2 groups did not differ in terms of the average length and rooting percentage. Seedling strength and health are related to the cutting type (Ke et al. 2001). Although the rooting percentages of cuttings with and without branches were identical, rooting occurred faster for cuttings with branches. Furthermore, the average root quantity was also larger with branches. These types of cuttings thus should be adopted for the water culture of *C. equisetifolia*.

All the above experiments demonstrated that the rooting percentage increases with rooting quantity. A higher rooting percentage

Table 4. Comparison of rooting quality with different wounding treatments

Rooting quality	Wounding treatment		*F*-value
	Wounding treatment (n = 90)	Non-wounding treatment (n = 90)	
Rooting percentage (%)	86.7±4.4	73.3±6.3	4.46**
Death percentage (%)	0.1	2.2±3.6	1.58^{ns}
Length (cm)	1.4	1.1	3.09*
Rooting number	2.2	1.3	6.70**

[1)] ^{ns} $p > 0.1$, * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$; Denote significant differences according to Duncan's multiple-range test.

[2)] Different letters indicate a significant difference of the means at the 0.05 level among detection methods by Duncan's new multiple-range comparison, mean±standard error.

[3)] Numbers in parentheses are percentages of the total.

Table 5. Rooting quality of different cutting types of *Casuarina equisetifolia*

Rooting quality	Cutting type		*F*-value
	Branch cuttings (n = 90)	Non-branch cuttings (n = 90)	
Rooting percentage (%)	87.8±5.3	87.8±5.3	0^{ns}
Death percentage (%)	0.1	0.1	0^{ns}
Length (cm)	1.1	1.3	1.278^{ns}
Rooting number	2.1	1.8	2.651*

[1] ns $p > 0.1$, * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$; Denote significant differences according to a Duncan's multiple test range test.

[2] Different letters indicate a significant difference of the means at the 0.05 level among detection methods by Duncan's new multiple-range comparison, mean±standard error.

[3] Numbers in parentheses are percentages of the total.

implies a larger rooting quantity. Ke (2001) studied the relationship between the rooting number and ratio of water-cultivated *C. equisetifolia* seedlings and showed that the rooting number was correlated (r = 0.935) with the rooting quantity. The goal of our research pertaining to *C. equisetifolia* was to collect and evaluate seed resources to identify a species suitable for the Taiwanese coast. Lou (2002) demonstrated that differences existed in rooting percentages both between and within populations. Furthermore, as rooting percentages are under the control of genetic selection, identification of suitable specimens is not impossible.

Casuarina equisetifolia is a widely distributed species of this genus. Both the morphology and genotype of these plants differ substantially depending on the environment (Ho et al. 2001). Such variations have been enhanced by the interbreeding among Taiwanese *C. equisetifolia* populations. A recent study of genetic variation in *C. equisetifolia* populations of Taiwan identified that *C. equisetifolia* naturally exhibits high levels of genetic variation and flexibility among widespread seed resources (Yang et al. 1995, Ho et al. 2002). Consequently seed resource experiments can provide a basis for improving the ability of *C. equisetifolia* to grow and thrive in coastal environments.

CONCLUSIONS

This study identified optimal conditions for the water culture of *C. equisetifolia*. The parameters obtained from this study can be expanded upon by further experiments which focus on additional factors that may affect rooting quality in water culture. Overall, the proficiency and standardization of water culture are necessary to establish a technique for the rapid and cost-effective generation of high-quality seedlings to meet the needs of renewing coastal forests in Taiwan.

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