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RESEARCH ARTICLE

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The optimizing conditions of soft-wood cutting of yam-lobation *Atractylodes macrocephala* Koidz.

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Abstract This study was focused on optimizing the conditions of soft-wood cutting for *Atractylodes macrocephala* Koidz., including the concentration and treatment time of IBA and IBA + NAA and the proportion of turf to perlite in substrates. $L_9(3^4)$ orthogonal design with three factors and three levels were adopted, and the best combination was chosen after formula analysis, variance analysis, and range analysis. The results showed that both the maximum survival rate and rooting rate were 93.33%. Statistic analysis showed that treatment with IBA ($200\ mg \cdot L^{-1}$) + NAA ($100\ mg \cdot L^{-1}$) for 20 s, turf to perlite ratio of 1∶3 would be the most favorable combination for soft-wood cutting of *A. macrocephala*.

Keywords *Atractylodes macrocephala* Koidz., soft-wood cutting, optimized conditions

1 Introduction

Atractylodes macrocephala Koidz. is a plant of the Chrysanthemum family (Guo, 2004), and its rhizoma is one of the famous materials of Chinese traditional medicine and one of the "Zhe Ba-wei" (Eight plant species used as the most excellent traditional medicines in Zhejiang Province, China). It has the efficacies of tonifying spleen and nourishing one's vitality, resolving dampness and inducing diuresis, consolidating superficial resistance, and antidiarrhea (Li, 1998). The main components of *A. macrocephala* were hinesol, Atractylon and Atractylodes (Duan et al., 2008). Modern pharmacological research showed that *A. macrocephala* had efficacies of diuresis, decreasing blood glucose and rallying leukocyte function after X-ray and chemotherapy.

At present, the main methods of planting *A. Macrocephala* are seeding and transplanting rhizomas. The modern vegetative propagation was mainly focused on tissue culture by using plant leaves and lateral buds (Peng et al., 2001; Chen et al., 2006; Hu et al., 2006; Zhu et al., 2006). However, it was not convenient to popularize the technique because of the technical complexity and the high cost of tissue culture. In this paper, to get the clones of plants, optimizing conditions for soft-wood cutting was studied including phytohormone treatment (concentration and time) and culture substrate (ratio of turf to perlite). The study was performed on two independent experiments by orthogonal design. The cutting materials were prepared from the plants with characters of dwarf and deep green leaves with the leaf shape of yams and disease resistance. This study would facilitate the propagation of *A. Macrocephala* plants with steady excellent character and provide superior plant resources for the Good Agriculture Practice (GAP) culture of *A. macrocephala*.

2 Materials and methods

2.1 Phytohormones and culture substrate

Phytohormones of IBA and NAA were purchased from Sigma company, and their solutions were prepared with the following concentrations and combinations: IBA $50\ mg \cdot L^{-1}$, $150\ mg \cdot L^{-1}$, and $300\ mg \cdot L^{-1}$ and also IBA + NAA, $(200 + 50)\ mg \cdot L^{-1}$, $(200 + 100)\ mg \cdot L^{-1}$, and $(200 + 150)\ mg \cdot L^{-1}$.

The substrates were prepared by mixing of turf and perlite at the ratios of 1∶1, 1∶2, and 1∶3 for soft-wood cuttings of *A. Macrocephala*. Moreover, the substrates

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were sterilized by 50% Carbendazim (800 ×) and covered with plastic film.

2.2 Plant cultivation

The marked yam-lobation *A. macrocephala* rhizomas were dug at the end of 2007 and deposited to keep them alive during the winter. In February 2008, similar and superior rhizomas without diseases and damages were chosen and soaked in 50% Carbendazim of 800 × for 10 h. Then, the rhizomas were transplanted into Baicaoyuan in Zhejiang Forestry University. The field was ploughed up at 25 cm depth, the diameter of soil particles was less than 1 cm, and manure was applied as base fertilizer. The seedbeds were 20 cm height and 100 cm width, and a space of 25 cm was taken between each other. The planting density was 20 cm × 30 cm, and the ditch was 10 cm depth. Plant ash of 6 cm depth was paved in the ditch, wherein five individuals were planted per row. Straw and flaccidness thatch were covered on the seedbeds to avoid the heavy rain and keep moisture in soil. Two months later, the epicormic branches were used for the experiments.

2.3 Experiment design

Two independent experiments using $L_9(3^4)$ orthogonal design (Ming, 2005) with three factors and three levels were performed in this study (Table 1).

2.4 Cutting

The *A. macrocephala* epicormic branches were obtained when the plants were 15–20 cm high in April 2008. In the experiments, the leaves were removed to decrease transpiration, and then, the segments were cut into 7-cm-length and placed for 5 minutes to make the insections a little dry. Thirty segments as a unit were treated following the experimental design. A glass rod was used to make holes of 2–3 cm in depth substrates to avoid scuffing the cortex of the segments' during planting. The segments were planting into substrate with the array of 5 cm × 10 cm. The substrate was compacted after planting.

A plastic shed was made over the planting area for heat and damp preservation (Table 2) and removed for ventilation at noon. The cuttings were water sprayed in the morning and evening twice a day. After 16 days of planting, the plastic shed was replaced by sun shading net whose transmittance of the sun light was 20%, and the cuttings were watered once a day to avoid stem decay.

2.5 Investigation, data statistics and analysis

Growth situations were checked every two days, including time of adventitious root formation (d), survival rate of wood cuttings (%), and the number of roots (per cutting).

The results were statistically analyzed by line grading law, formula analysis (Yuan, 2005), variance analysis, and range analysis. The formula was as follows:

$$\text{Integrated grade} = 5 \times \text{rooting rate grade} + 3 \times \text{mean root number grade} + 2 \times \text{rooting time grade.}$$

3 Results

3.1 Treatments results of IBA and IBA + NAA

Orthogonal experiment results (Table 3) showed that all soft-wood cuttings of *A. macrocephala* treated with the two phytohormones rooted. In the experiment I, both the highest rooting rate and survival rate among the treatment of No. I-7 were 80.0%. The rooting time varied depending on different treatments. There was a phenomenon of pseudosurvival (pseudosurvival rate = viability − rooting

Table 1 Design of $L_9(3^4)$ orthogonal experiment

level	factor			level	factor		
	A/(mg·L^{-1})	B/s	C		A/(mg·L^{-1})	B/s	C
I-1	50	10	1:1	Π-1	200 + 50	10	1:1
I-2	150	20	1:2	Π-2	200 + 100	20	1:2
I-3	300	30	1:3	Π-3	200 + 200	30	1:3

Note: A: concentration of phytohormones, B: treatment duration, C: substrate proportion.

Table 2 Environmental temperature and humidity

recording time	average temperature/°C			average humidity/%		
	highest	lowest	average	highest	lowest	average
April 23–May 8	26.7	11.5	19.2	95.5	86.3	90.4
May 9–May 23	29.1	14.3	22.6	87.9	71.6	79.2
May 24–June 7	31.9	20.6	25.3	94.3	67.7	75.7

Table 3 Orthogonal experiment results of the rooting of soft-wood cuttings of *A. macrocephala*

treatment	rooting time/d		rooting rate/%		viability/%		average root number /(root·plant^{-1})	
	I	II	I	II	I	II	I	II
1	22	28	43.3	63.3	50.0	70.0	11.7	17.3
2	26	24	63.3	76.7	63.3	86.7	15.0	16.7
3	22	24	70.0	80.0	73.3	83.3	19.3	13.3
4	26	24	56.7	86.7	60.0	86.7	18.3	20.0
5	19	20	76.7	93.3	80.0	93.3	23.7	26.3
6	26	28	53.3	63.3	66.7	66.7	12.0	12.7
7	22	24	80.0	76.7	80.0	86.7	26.3	16.7
8	26	28	53.3	53.3	56.7	60.0	13.7	7.3
9	26	28	73.3	63.3	73.3	70.0	16.7	11.0

rate) whose rate was 3.3%–13.3%. The results showed that the average rooting rate (58.9%, 62.2%, and 68.9%, respectively) and the average root number (15.3, 18.0, and 18.9, respectively) were increasing depending on the increasing concentration of IBA. All soft-wood cuttings of *A. macrocephala* could root in different substrates. The average rooting time was reduced (24.7 d, 26.0 d, and 21.0 d, respectively), and the average rooting rate (50.0%, 64.4%, and 75.5%, respectively) and the average root number (12.5, 16.7, and 23.1, respectively) were increasing with the ratio of turf to perlite.

In experiment Π, both the rooting rate and survival rate were the highest (93.3%) under the condition of 200 mg·L^{-1} IBA + 100 mg·L^{-1} NAA, during 20 s treatment and at 1∶3 ratio of turf to perlite. The average rooting rate was 73.3%, 81.1%, and 64.4%, respectively, and the average root number was 15.8, 19.7, and 11.7, respectively, both of which increased with the reducing of the concentrations but decreased with higher concentrations of IBA + NAA. There was also a phenomenon of pseudo-survival, and its rate was 3.33%–10.00%. The average rooting duration reduced to 26.7 d, 24.0 d, and 22.7 d, respectively, with increasing ratio of turf to perlite, but the average rooting rate was increased to 60.0%, 75.6%, and 83.3%, and the average root number was increased by 12.4, 15.9, and 18.8, respectively.

In these two experiments, the results showed that the treatment of 10 s and 20 s made earlier rooting and higher average root number than that of 30 s.

3.2 Statistical analysis of IBA and IBA + NAA treatments

The rooting rate was the most conclusive index in cutting experiments; however, other assistant indexes, such as mean root number and rooting time, should be taken into account for optimizing the conditions. Line grading law analysis and formula analysis were used in this research (Table 4).

Table 4 shows that the grade of No. I-7 was the highest with the condition of soaking in 300 mg·L^{-1} IBA for 10 s, and the turf to perlite ratio was 1∶3 in experiment I. The variance analysis results showed that there was a significant difference of factor C (F = 25.487, $F_{0.05}$ = 19.00) in the confidence interval. The grade of No. Π-5 was the highest in experiment Π. The results of variance analysis showed that there were significant differences between factor A (F = 26.399, $F_{0.05}$ = 19.00) and factor C (F = 45.776) in the confidence interval.

Statistic analysis results (Table 5) showed that the gradation of the factors was C > A > B in the two

Table 4 Formula analysis of experimental index grade

treatment	rooting time		rooting rate		mean root number		integrated grade	
	I	II	I	II	I	II	I	II
1	12.3	2.0	5.0	16.3	3.0	17.2	20.3	35.5
2	2.0	11.0	29.5	31.3	9.1	16.3	40.7	58.5
3	12.3	11.0	37.7	35.0	17.1	11.5	67.1	57.5
4	2.0	11.0	21.4	42.5	15.3	21.0	38.6	74.5
5	20.0	20.0	45.9	50.0	25.1	30.0	91.0	100.0
6	2.0	2.0	17.3	16.3	3.6	10.6	22.9	28.8
7	12.3	11.0	50.0	31.3	30.0	16.3	92.3	58.5
8	2.0	2.0	17.3	5.0	6.7	3.0	26.0	10.0
9	2.0	2.0	41.8	16.3	12.2	8.2	56.0	26.5

Table 5 Range analysisof the formula grade

average value	IBA				IBA + NAA			
	A	B	C	index grade	A	B	C	index grade
k_{j1}	42.7	50.4	23.0		50.5	56.2	24.8	
k_{j2}	50.8	52.6	45.1	$\sum = 151.6$	67.8	56.2	53.2	$\sum = 149.6$
k_{j3}	58.1	48.7	83.5		31.7	37.6	72.0	
R_j	15.4	3.9	60.4		36.1	18.6	47.3	
best combination	A_3	B_2	C_3	$A_3B_2C_3$	A_2	B_2	C_3	$A_2B_2C_3$

Note: A, B, and C represented factor;1, 2, and 3 represented level; k_j representedaverage value of factor and level; R_j represented range value.

experiments. It was the most optional condition for the soft-wood cutting of *A. macrocephala* of $A_3B_2C_3$ (300 mg·L^{-1} IBA, for 20 s, at 1∶3 ratio of turf and perlite) in experiment I, and 200 mg·L^{-1} of IBA + 100 mg·L^{-1} of NAA, for 20 s of treatment at 1∶3 ratio of turf, and perlite was the best condition for soft-wood cutting of *A. macrocephala* in experiment Π.

4 Discussion

4.1 Mechanism of adventitious root formation for the soft-wood cutting of *A. macrocephala*

There were two types of root formation for the soft-wood cutting (Bai et al., 2003), including the latent and primordial root anlage, namely, phloem root formation and the primordial root induced *in vitro*, namely, incision root formation. The callus tissues appeared initially in all kinds of *A. macrocephala* cutting woods in different treatments, and then, the callus tissues were differentiated into roots. Because there was no root formation from the phloem in the soft-wood cuttings of *A. macrocephala*, it belonged to the second rooting type. The rooting mode of *A. macrocephala* soft-wood cutting was reported for the first time.

4.2 The effects of phytohormones and substrate on the root initiation in *A. macrocephala* soft-wood cuttings

Both NAA and IBA could induce rooting (Wu et al., 2001; Xu et al., 2004), but the roots induced by IBA were only slim and long, therefore, IBA was often mixed with NAA in production (Yang and Wang, 1999). In our experiments, the results from the treatments with both IBA and NAA together showed well additive effects (Van der Krieken et al., 1993), which was beneficial to *A. macrocephala* rooting. The results also showed that the 1∶3 ratio of turf to perlite was the best proportion for rooting, because the substrate proportion provided good water-holding and breathable conditions for soft-wood cutting of *A. macrocephala* (Zhang and Wang, 2008).

The most promising treatment of soft-wood cutting of *A. macrocephala* was to soak the cuttings in IBA (200 mg·L^{-1}) + NAA (100 mg·L^{-1}) for 20 s, at 1∶3 ratio of turf to perlite.

4.3 Phenomenon of pseudosurvival

There was a phenomenon of pseudosurvival (Liu, 2001) in which the seedlings had callus tissues only without root formation. If adventitious roots could not develop from the callus tissues, the *A. macrocephala* cutting woods would become pseudosurvival seedlings and lead to death finally (Li et al., 2003). Therefore, decreasing the pseudosurvival rate was the most important for enhancing the survival rate of *A. macrocephala* cuttings.

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