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Research of Factory Guarantee for Acacia Melanoxylon Clonae

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Abstract

Acacia melanoxylon is a kind of new fast-growing tree specie incorporating high economical, ecological and social benefits, having the high value for popularization. In this paper, we discussed the procedure of tissue culture and rapid propagation of Acacia melanoxylon, such as getting the explants, inducing axillary bud, the redifferentiation of callus, multiplying the caespitose shoots and strengthening the seedling roots. The technology for massive production of cloning Acacia melanoxylon was established. The result showed that it was better to disinfect the explants for one minute with 75% alcohol and then for 15 minutes with mercury. BA0.5-1 mg.L-1+ NAA 0.5 mg.L-1+ IBA 0.5 mg.L-1 was the best culture medium for inducing callus whose rate of succeeding in inducing was up to 96.7%.6-BA 1 mg.L-1+IBA 0.5 mg.L-1 was the best culture medium for the redifferentiation of callus. 6-BA0.5~1mg.L-1+IBA0.1mg.L-1 was the best culture medium for multiplying. 1/2MS+IBA0.5~1.5 mg.L-1+NAA0.5 mg.L-1 was the best culture medium for the seedling roots. In the experiment, the sterile segment with axillary bud was set as material, the high-effective and fast-speed sterile reproduction system was successfully built through adjusting the hormone combination of culture medium in ex-plant induction, proliferation, and callus redifferentiation process, achieving the planting process from ex-plant acquisition to seedling & transplantation of the acacia melanoxylon, and providing the theoretical support for large-scale and industrial production of acacia melanoxylon.

Keywords: Acacia Melanoxylon; Tissue Culture; Stroma; Rapid Propagation; Cultivation

INTRODUCTION

Acacia melanoxylon, a kind of evergreen arbor, belongs to acacia mill of leguminosae mimosaceae as one of the tallest arbors among acacia species. It is featured with fast-growing, high-yield and strong adaptability, nodulated and nitrogen-fixed, strong resistance to hydrogen, sulfur dioxide and chlorine gas, as well as abundant litter falls and good soil modification property. As preferable tree species for industrial raw wood in short period and tree species of ecological protective forest, the Acacia melanoxylon is good for soil improvement, increase of soil productivity and soil & water conservation, capable of being used for greening in polluted area. With the beautiful pattern and good texture, it boasts extremely high value in application on furniture and laminated wooden board; besides, it is high-quality materials for the backboard of violin due to the excellent acoustic performance. The current market price of Acacia melanoxylon raw wood is about RMB 3000/m^3, tending to increase constantly (Liu Li 2015). As a newly introduced tree species in China, the Acacia melanoxylon incorporates high economical, ecological and social benefits.

The rapid propagation technology of acacia melanoxylon has already been reported (Meyer et al.1987; Su Jingqiang et al. 2008; Wang Panpan et al.; Lin Laishui; Luo Wanye et al. 2006). Huang Liejian et al. (2015) used growth regulators for induction of proliferation of axillary bud, however, the callus grew excessively, with low proliferation multiples and poor seedling quality. Dai Zhiming et al. (2012) achieved the axillary bud proliferation with the sprout segments of seed, but cannot ensure the quality of plants. In order to avoid excessive callus and poor growing condition of bud during rapid propagation of acacia melanoxylon, through adjusting the types and concentration of

growth regulations in culture medium, the rapid propagation of acacia melanoxylon with preferable seed and fine property was built in this paper, providing scientific basis for industrial production.

MATERIALS AND METHODS

Material

The experimental species were Acacia melanoxylons supplied by the national fine acacia variety base. The stem segments of healthy and strong Acacia melanoxylon of new branches were taken as ex-plant materials.

Method

First-generation Culture of Acacia Melanoxylon

Terminal buds, axillary buds and other tissues of Acacia melanoxylon are intended to be highly polluted. The most effective disinfecting method can not only make the experiment result more accurate, but also provide factory guarantee for Acacia melanoxylon clonal series (Luo Wanye et al.2006). Firstly, the terminal buds were cleaned with powdered detergent, and then the bud body leaves were removed and placed in flowing waters for flushing. Finally, the following six methods were used for disinfection with observation and comparison of effect.

As for the first method, the test piece will be placed in 0.1% mercuric chloride solution and maintained for 10min. As for the second, third and fourth method, the test piece will be added in 75% alcohol and kept for 1min and then washed with sterile water and processed by 0.1% mercuric chloride for 9min, 12min and 15min. As for the fifth and sixth method, the test piece will be added in 75% alcohol and kept for 1min and then washed with sterile water and processed by 5% calcium hypochlorite for 15min and 20min respectively. After the treatment of above all methods, the sterile water will be used for washing for three times with each for not less than 1min, then the moisture of bud body will be dried, and then inoculated to the induce culture medium.

Induction for Adventitious Bud of Ex-plant

The axillary buds sprouted from terminal buds and stem segments were mainly used for induction of ex-plant in the experiment. Configuration of induce culture medium: to know the effect of different configurations of growth regulator on induction of ex-plant, the improved MS culture medium with 30 g.L-1 sucrose is added with BA, NAA and IBA. The following 10 types of culture media were combined as shown in Table 1. The PH value is at 6.5.

TABLE 1 DIFFERENT GROUP OF GROWTH REGULATOR COMBINATIONS AND CONCENTRATION RATIO

Group	Different configurations of growth regulator
1	BA0.5+NAA0.5+IBA0.5
2	BA0.5+NAA0.5+IBA1
3	BA0.5+NAA1+IBA0.5
4	BA0.5+NAA1+IBA1
5	BA1+NAA0.5+IBA0.5
6	BA1+NAA0.5+IBA1
7	BA1+NAA1+IBA0.5
8	BA1+NAA1+IBA1

Successive-Generation Culture

The pollution-free, browning and vitrificated ex-plant were transferred to the successive-generation culture medium for induction of callus and adventitious bud. The clustered buds would be transferred to the successive-generation culture medium for reproduction after it was cultured by induction culture medium to about 5cm. In successive-generation culture medium, the bud primordium was formed at the base of bud and them developed into bud cluster for reproduction, in order to know the effect of successive-generation times to reproductive culture of tube seedling, and to make bud and seedling grow in healthy plants as soon as possible and to provide the best bud for reproduction. The seven combinations（A: 6-BA0.5mg.L-1+NAA0.5mg.L-1; B: 6-BA1mg.L-1+NAA0.5mg.L-1; C: 6-BA0.5 mg.L-1+IBA0.1 mg.L-1; D: 6-BA1 mg.L-1+IBA0.1 mg.L-1; E: 6-BA0.5 mg.L-1+IBA0.5 mg.L-1; F: 6-BA1 mg.L-1+IBA0.5 mg.L-1） based on the MS culture medium were used for comparison experiment, and the result after

culture for 93d was shown in Table 5.

Rooting Culture of Bud/Seedling

The rootless seeding, after being reproduced rapidly in reproductive culture medium to about 2-3cm, would be cut into single seedling and then transferred into the rooting culture medium, in which 1/2 MS would be set as the basic culture medium to induce the rooting of bud/seedling. Next, the bottle seedling would be transferred to the shaded environment in natural light and kept for 1-2 weeks to let it adapt the natural light as soon as possible.

In the experiment, the 1/2 MS was taken as basic culture medium and the IBA and NAA as the tested factors for the comparison test; besides, the setting concentrations were IBA: 0.5mg.L-1, 1 mg.L-1, 1.5 mg.L-1 and 2 mg.L-1, and NAA: 0.5 mg.L-1 and 1 mg.L-1 respectively. The result after culture for 30d was shown in Table 5.

TABLE 5 EFFECTS OF DIFFERENT GROWTH REGULATOR COMBINATIONS ON MULTIPLICATION OF INDUCED BUDS

Group	Stem segment number	Proliferation times	Growing situation
A	50	1.91e	The calluses become more and their color is mainly white while partly light green. The proliferous buds become small and dense.
B	50	2.24d	The calluses become larger and their color is light green. Part of explants are covered with calluses. The proliferous buds become small and slightly vitrified.
C	52	3.15b	The proliferous buds become small but have a strong germination. There are many sprouts and a few calluses.
D	50	3.35a	The proliferous buds and calluses become more.
E	53	2.26d	There are many thin proliferous buds and a few calluses.
F	50	2.75c	There are many thin proliferous buds and many sprouts. The callus is loose.

Note: Superscripts represent significant difference at 0.05 level with LSD multiple analysis（$P<0.05$）.

Culture cCondition and Transplantation Management

In bottle-seedling period of tube seedling, the test materials would be put into artificial climate chamber for culture after being transplanted successfully. In first-generation and successive-generation culture stages, the temperature would be controlled at 24-26℃, light intensity at 1000-1500lux, and light duration of 10h per day (Huang Liejian et al.2015). In rooting and hardening-off stages, the temperature in culture room shall not exceed 34℃, and natural light would be strengthened to make the tube seedling grow under approximately natural environment.

In order to improve survival rate of buds in seedling emergence and transplantation stages, the experiment base should be completely disinfected before transplantation. The rooted seedling with root about 0.5cm would be selected with culture medium cleaned off after hardening-off, and then the seedling would be transplanted in disinfected medium and irrigate with rooting water in proper amount after its root was disinfected. The sunshade net can be removed after shaded culture for 5-7d, and the seedling can be managed by normal water and fertilizer.

RESULTS AND DISCUSSION

Impact of Different Disinfection Methods on Culture of Ex-plant

The terminal buds of new branches of Acacia melanoxylon picked in early summer were disinfected after the terminal buds were removed. The results （Table 2）showed that although the ex-plant processed only with mercuric chloride has low browning rate, the relative pollution is much greater. Moreover, if the ex-plant was processed by 75% alcohol and then the survival rate would be identically improved while the browning rate was relatively increased within 9-15min during being processed with mercuric chloride. The effect of disinfection treatment with hypochloric acid for 15~20min was similar to the treatment with mercuric chloride for 15 minutes. By contrast, the treatment with mercuric chloride for 15min after the 75% of alcohol for 15 minutes leaded to the relatively good effect, having the survival rate reaching to 55.8%.

TABLE 2 EFFECTS OF DIFFERENT METHODS OF DISINFECTION EFFECT OF EXPLANTS

Group	Stem segment number	Pollution number	browning number	survival rate%
1	50	41	6	18.0e
2	47	30	12	34.0d
3	52	23	13	46.2c
4	52	20	15	55.8a
5	48	21	10	50.0b
6	51	20	15	52.9a

Impact of Different Growth Regulators on Callus Induction

By connecting the Acacia melanoxylon stem segments with axillary buds to the culture medium, and after a week, there was callus generated at the base of stem segments. The research tested the impact of different growth regulators on adventitious buds of Acacia melanoxylon. It can be seen from Table 3 (ratio of different culture media and effect of callus induction) that: under the condition where the NAA concentration was 0.5 mg.L-1 and IBA0.5 mg.L-1, the callus induction ratio of BA under the concentration of 0.5 mg.L-1 and 1 mg.L-1 was higher than 90%. With the increase of the concentration of the three, the callus induction ratio reduced and when the concentration of either two types was 1 mg.L-1, the callus induction ratio was lower than that when the concentration of any type was 1 mg.L-1. The callus induction ratio was the lowest when the concentration of the three reached 1 mg.L-1. Therefore, the growth regulator suitable for the callus induction of Acacia melanoxylon was BA0.5~1 mg.L-1+ NAA 0.5 mg.L-1+ IBA 0.5 mg.L-1.

TABLE 3 EFFECTS OF DIFFERENT GROWTH REGULATOR COMBINATIONS ON INDUCTION OF CALLUS

Group	Stem segment number	Germination days	Callus number	callus induction rate	Growth
1	30	20	29	96.7a	+++
2	29	17	26	89.7c	++
3	33	16	27	81.8d	++
4	32	17	26	81.2d	++
5	35	15	32	91.4b	+++
6	31	15	24	77.4f	+++
7	30	18	24	80.0e	+++
8	30	15	12	40.0g	+

Note: +，least promoting effect，+++，maximum promoting effect.

Impact of Different Growth Regulators on Callus Re-differentiation

It can be found from tests that clustered buds of Acacia melanoxylon can be obtained by using callus re-differentiation besides axillary bud induction. In Table 4, the impact of different media components on callus re-differentiation of Acacia melanoxylon was reflected through testing the re-differentiation ratio of callus impacted by different growth regulators. From Table 4, it can be seen that the re-differentiation ratio of callus in the culture medium with IBA was higher than that in the culture medium without IBA, and that in the culture medium with 6-BA 1 mg.L-1+IBA 0.5 mg.L-1 is the highest.

TABLE 4 EFFECTS OF AUXINS ON RE-DIFFERENTIATION OF CALLUS

Group	Stem segment number	re-differentiation number	re-differentiation rate
A	60	32	53.3d
B	59	32	54.2d
C	62	55	88.7c
D	60	57	95.0b
E	62	60	96.8a
F	63	62	98.4a

Impact of Different Growth Regulators on Bud Propagation

It was showed that the clustered bud propagation of Acacia melanoxylon had the most obvious effect with strong germination and good callus growth if MS was the main culture medium and the growth regulators were 6-

BA0.5mg.L-1, IBA0.1mg.L-1 combination and 6-BA1mg.L-1, IBA0.1mg.L-1 combination. When IBA was replaced with NAA, callus increased or enlarged, but the bud color became light and the bud vitrified. When the concentration of IBA reached 0.5mg.L-1, the clustered buds looked like long and thin and the callus was loose.

Different Culture Media on Rooting of Bud Seedling

It can be seen from Table 6 that, in the test of rooting rate of Acacia melanoxylon induced by IBA and NAA combination with 1/2MS as the main culture medium, with the increase of the concentration of IBA, the rooting rate increases and the root system of rooted plantlets was sturdy and the seedling grows well under the condition where the concentration of NAA was 0.5mg.L-1,IBA≤1.5 mg.L-1; the rooting rate started to decrease, the callus formed reduced and rooted plantlets became weak when the concentration of IBA reached 0.5mg.L-1. If the concentration of NAA was 1mg.L-1, the rooting rate was above 80%, but the seedling grew weak, and although there were many root systems, the roots were fine and short. The culture medium most suitable for rooting of Acacia melanoxylon in the treatment test was 1/2MS+IBA0.5~1.5 mg.L-1.

TABLE 6 EFFECTS OF DIFFERENT GROWTH REGULATOR COMBINATIONS ON ROOTING OF ACACIA MELANOXYLON

Culture medium	Plantlet number	Rooting number	Rooting rate	Growth
1/2MS	30	13	43.3	+
1/2MS+IBA0.5+NAA0.5	30	26	86.6	+++
1/2MS+IBA1+NAA0.5	30	28	93.3	+++
1/2MS+IBA1.5+NAA0.5	32	31	96.9	+++
1/2MS+IBA2+NAA0.5	32	29	90.6	++
1/2MS+IBA0.5+NAA1	30	26	86.7	++
1/2MS+IBA1+NAA1	30	24	80.0	++
1/2MS+IBA1.5+NAA1	32	28	87.5	+
1/2MS+IBA2+NAA1	30	26	86.7	+

The more of +, the better of bud growth.

Transplanting Methods of Acacia Melanoxylon

Transplanting Conditions of Acacia Melanoxylon

Tissue culture seedlings shall be transplanted at the right moment after growing to a certain degree. Healthy sprouts free of diseases were selected from tissue culture seedlings of Acacia melanoxylon. The general standard was that the height of plant was about 2.5m and there was at least one rhizome and leaves had become green (Li Zhixian 2010; Wen Weiwen et al. 2013). The best transplanting season was spring and transplanting of tissue culture seedlings in spring would greatly increase the survival rate. Pay attention to the depth of sprout buried in the soil during transplanting. If it was too shallow, it would not be good for rooting and soil nutrition absorption of Acacia melanoxylon; if it was too deep, heartrot would be caused.

Transplanting Environment of Acacia Melanoxylon

Light

Acacia melanoxylons liked to be exposed to sun and were strong positive species. During cultivation of tissue culture seedlings, the light intensity shall be controlled to be 4000~5000lux and temperature 25±2℃. The light intensity of hardening seedlings shall be 5000~8000lux. After seedlings were moved out of bottles, a field with full light and long exposure time to sun shall be chosen.

Humidity

Acacia melanoxylon is resistant to drought and has low requirements for moisture. In the period of frequent rainfall, artificial watering is almost unnecessary, and also it shall be avoided that the Acacia melanoxylon is flooded. A field with a good drainage performance shall be selected as too much rainfall may cause ponding or over-high humidity which may easily cause rotted roots.

Temperature

Acacia melanoxylon is a tropical and subtropical plant and the highest temperature it can endure is 40℃ (He Minghui 2015). It can also endure mild hypothermia and does not suffer frost. A forest land with warm climates is good for Acacia melanoxylon to adapt to the transplanting environment more quickly and promote rapid growth.

Rational application of fertilizer

At the initial stage of seedling transplanting, the seedlings were fragile and easy to be damaged due to interference of external factors (Han Jinfa 2006). Therefore, the surrounding weeds shall be removed and fertilizer is used to promote the growth. The first fertilization shall be carried out in the second month after seedlings are transplanted and then the follow-up fertilization shall be conducted according to the growth.

CONCLUSION

In the research, branches of good sources of Acacia melanoxylon were treated as explant materials to establish an efficient and rapid propagation system of Acacia melanoxylon clone tissue culture. And the system was cultivated for 20d on the culture medium with the rooting rate of 96.9%. The Acacia melanoxylon obtained through the research had overcome the disinfection difficulty of Acacia melanoxylon explant, having effectively ensured fine properties to make the Acacia melanoxylon clone system applied to industrial production. To improve the rapid propagation system of Acacia melanoxylon, the following items will be carried out in the future:

(1) Continue to optimize test media and further achieve the rapid propagation of Acacia melanoxylon with the method of culture medium material and ratio adjustment.

(2) Improve tissue culture seedlings of Acacia melanoxylon to make them grow healthily in various environments, fight against various plant diseases and insect pests and improve the growth speed of Acacia melanoxylon and lumber quality.

(3) Achieve a larger-scale and more standard planting industry of Acacia melanoxylon and improve the survival rate and merchant ratio of seedlings.

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