Regeneration of Blue Honeysuckle via Dormant Axillary Buds

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Abstract: The optimum medium for dormant axillary buds culture of blue honeysuckle was screened according to the growth rate and elongation rate by inoculating the buds on culture medium with various 6-BA and iron-salt concentration. About 35 days, the stretched stem buds were divided into strong root system after inoculated on 1/2 MS+1.0 mg·L^{-1} IBA rooting medium. Amount of qualified tissue-cultured young plants could be obtained by the stretched stem buds reproduction.

Key words: blue honeysuckle, tissue culture, axillary buds

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Introduction

Blue honeysuckle is a kind of wild berry plant belonging to *Lonicera* genus, Caprifoliaceae family, mainly distributed in Russian, China, Japan, North Korea and North America. The species of plant contain high content of Vitamin P (VP) and flavonoid compounds. The highest content of VP can reach 28 mg·g^{-1}[1]. Blue honeysuckle is also an extremely good resource for natural dietary pigment production because the content of anthocyanidin in its fruits is up to 3.28-6.21 mg·g^{-1}[2]. Recent years, Russia, Japan and China have done some researches on the variety introduction and breeding[3-6]. Blue honeysuckles reproduced with vegetative propagation are prone to have variability in their seedling progeny. For obtaining a great volume of qualified young blue honeysuckles seedlings, tissue culture is used to yield many qualified young plants in short time when mother plants resources are not so many. This is also a foundation for potential mass plantation.

Materials and Methods

Explant treatments

Two genotypes, *L.caerulea* var. *edulis* and *L.caerulea* var. *altaica* were studied in this experiment. The twigs were cut into small segments between internodes, processed with 75% alcohol for 5 min followed with 0.1% mercury liquid treatment for 3-5 min. Picked up the meristem tips with anatomical needle after washing the stem segments using sterile water. The tips were cut off and inoculated on solid medium, maintaining the temperature in the range of (25±2)℃ and retaining 16 h of light periodic culture.

Germination culture medium of axillary buds

The medium for axillary buds germination was classified into (A) MS+1 mg·L^{-1} 6-BA, (B) MS+2 mg·L^{-1} 6-BA, (C) MS (double content of iron-salt)+1 mg·L^{-1} 6-BA and (D) MS (double content of iron-salt)+2 mg·L^{-1} 6-BA. All of them were supplemented with 3% sugar, 0.7% agar powder and kept the medium pH 5.7. Vacci-

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nated 5-8 buds in per culture bottle of 50 mL. Repeated the same process three times and three bottles at each time.

Rooting and regenerating

When the buds generated and young leaves grew up to certain height, they were transported into rooting medium of 1/2 MS+1.0 mg·L^{-1} IBA. When the young shoots developed to 5 cm high, stem nodes with leaves were cut off and inserted into rooting medium.

Results and Analysis

Effects of the content of 6-BA and iron-salt on axillary buds germination and elongation

The effects of growth rate (germination number of shoot tip/total amount of axillary shoot tips×100%) and elongation rate (number of shoot tips longer than 2 cm/total amount of germination tips×100%) of meristem tips deprived from axillary buds without germinating were statisticalized after 30 days when the tips were inoculated on four culture medium A, B, C and D. The results are shown in Fig. 1.

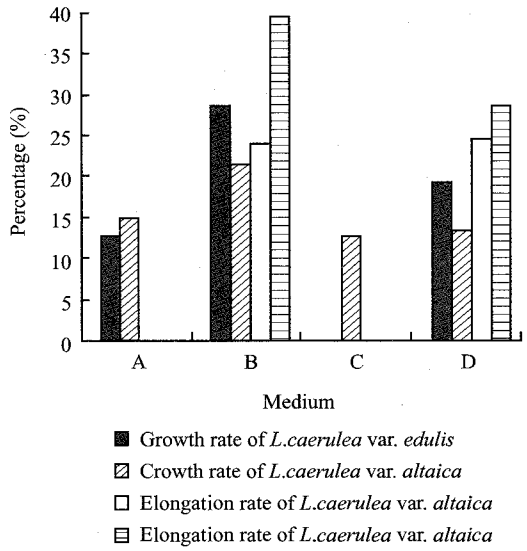
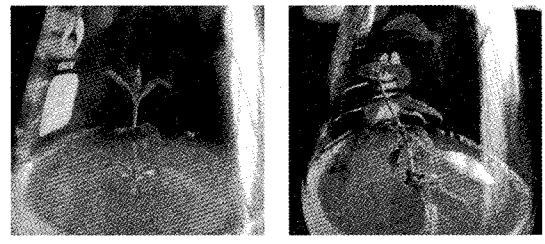


Fig. 1 Effect of different culture medium on the growth and elongation of axillary buds of blue honeysuckle

The plants, cultured in the medium B with the content of 2 mg·L^{-1} 6-BA, were obviously better performed than that grew on others. They were strong and grew rapidly. Though meristem tips also can perform high growth and elongation rate when the concentration of iron-salt was doubled with 2 mg·L^{-1} culture medium, cultivated buds grew slowly and the meristem tips had the tendency of getting brown and necrosis. In culture medium A and C with 1 mg·L^{-1} 6-BA, the plants growth rate and elongation rate of meristem tip were lower. Furthermore, the top of the buds appeared necrosis. MS culture medium supplemented with 2 mg·L^{-1} 6-BA and common iron-salt content were contributed to the growth and elongation of meristem tips. Two different genotypes, *L.caerulea* var. *edulis* and *L.caerulea* var. *altaica*. had almost the same cultivation standard.

Root development of cultivated young plant and regeneration

After 30-40 days, when the height of the buds grew up to 2-3 cm, cut off at least a pair of leaves on the buds with sterile sharp scissor, inserting the meristem tips into a rooting medium with combination of 1/2 MS+ 1.0 mg·L^{-1} IBA. The growth state in the rooting medium for 6 days is shown in Fig. 2A. After cultivated on rooting medium for about 35 days, the axillary buds, as shown in Fig. 2B, had yielded many notes and leaves which were robust with well-performed root systems and new germinated axillary buds.



A 6 days B 35 days

Fig. 2 Tissue-cultured blue honeysuckle growing on rooting medium for 6 and 35 days

During one month, the plantlets were formed after taking a second cut of the new shoots and inserting them into new medium. The leftover mother plants

sprouted fresh batch of lateral buds from basal leaves, because of loosing meristem tips and apex dominance. Thus, many qualified young plants can increase rapidly in the pattern of index.

Discussion and Conclusion

The advantages of utilizing axillary buds as the material for rapid propagation are followed: first of all, materials for propagation are abundant, which have merit for regeneration of precious species as every twig yields many axillary buds. Secondly, dormant buds can be easily transported. The good example of not affecting the later tissue culture steps is the long distance transportation of dormant twigs under moisture from Harbin to Beijing for this study. Material can be kept in high germination state even after preserved 1-2 months at 4℃.

This research analyzed the effects of culture medium on the growth and elongation rate from two aspects, concentration of 6-BA and iron-salt. Medium B and D stimulated the growth of axillary buds, medium A and C supported prior bud growth compared with the previous two media. It illustrated that the growth and elongation rate of dormant bud tips were sensitive to 2 $mg \cdot L^{-1}$ medium. High iron-salt content would lead to the necrosis of the tip while standard iron-salt concentration in MS media contributed to the growth of the bud. This result consensuses with that of Karhu[7-8].

Blue honeysuckle, as a shrub, has a good character of root development. Plants will sprout thick lateral roots after young leaves grew in rooting medium for about one month, meanwhile the growth volume of mother plants can also be very predominant, which is beneficial for rapid propagation.

In brief, technical assurance of tissue-cultured plants for the propagation of blue honeysuckle which is regarded as an economic berry is provided by the optimized growth and elongation medium through the systemic research of root development.

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