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EFFECTS OF CULTURE TEMPERATURE ON MICROTUBER FORMATION OF *ACONITUM CARMICHAELII* DEBX. AND ACONITINE-TYPE ALKALOID CONTENTS

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SHIPING C., SHAN S. J, TANAKA H. and SHOYAMA Y. *Effects of culture temperature on microtuber formation of Aconitum carmichaelii Debx. and aconitine -type alkaloid contents* BIOTRONICS **27**, 15–20, 1998. The clonally propagated *Aconitum carmichaelii* Debx. was used for the investigation of microtubering. The rooting condition of propagated shoots affected the establishment of transplantation to soil indicating the Murashige-Skoog medium supplemented with 0.5 mg/l of IAA and the cultivation at 20℃ are the best. Culturing at 15℃ under the dark enhanced microtubering rather than 10℃ or 20℃. The temperature affected the production of aconitine-type alkaloids, demonstrating that the contents of mesaconitine and hypaconitine were higher at 20℃ than at 15 and 10℃.

Key words: *Aconitum carmichaelii*; tissue culture; microtuber formation; aconitine-type alkaloids; temperature dependent.

INTRODUCTION

The tuber of *A. carmichaelii* Debx., a perennial herb of the family Ranunculaceae indigenous to China, has been used as one of the most important Chinese drugs, "Fu-tzu" or "Fu-pen", prescribed together with other herbal drugs, as an analgesic in the treatment of rheumatism and neuralgia. It is, however, dangerous to use the crude drug since the range between a therapeutic dose and a toxic dose is quite narrow, especially due to the aconitine-type alkaloids such as aconitine, hypaconitine and mesaconitine. It is well-known that the crude drug contains many kinds of pharmacologically active alkaloids, like higenamine (*1*), coryneine (*2*), benzoylaconine-type alkaloids (*3, 4*) and lipoaconitine-type alkaloids (*5*). The significant differences in the type and quantity of alkaloids have been observed depending on the place of growth and the season (*6*). In order to breed a homogeneous strain of *A. carmichaelii* with respect to the quality and quantity of aconitine-type alkaloids, we have already reported the clonal micropropagation of *A. carmichaelii* by shoot tip culture and the subsequent restoration of adult plants and short-term-cultivation for the

analysis of alkaloids (*7*), and also somatic embryogenesis via anther culture (*8*).

On the other hand, *A. carmichaelii* functions as an important ornamental flower in Japan. However, since the daughter tubers have been cultivated by division repeatedly, the problems of pathogen infection offen occur. Establishment of regenerated plantlets to soil is the most important stage in plant tissue culture projects. Moreover, we previously determined that the propagation ratio of this plant was 6 times in a year in the field cultivation (*9*). Therefore, rapid and simple propagation method is needed for the practical cultivation for the flower production. In this communication, we describe the effect of culturing temperature on the establishment of transplantation to soil, the microtuber formation from the clonally propagated shoots and the aconitine-type alkaloid content.

MATERIALS AND METHODS

Plant material

Clonal *A. carmichaelii* plants were produced by shoot tip culture as previously reported (*7*). Ten propagated shoots were cultured for rooting on Murashige-Skoog (MS) (*10*) medium supplemented with varing concentrations of IAA (0 to 2 mg/l) in 16 hr light from cool white fluorescent tubes (2000 lux) at 20 ± 1℃ for 4 weeks. Ten rooted plantlets were transplanted to vermiculite and cultivated at 20 ± 1℃ in the phytotron of Kyushu University for 8 weeks in 1997 (Fig. 1-A; Table 1).

Ten rooted plantlets were successively cultured under the dark condition in the growth cabinet at 10 ± 1, 15 ± 1 and 20 ± 1℃ for 6 weeks (Fig. 1-B; Table 2). Microtubers were divided individually, stored in a refregerator (4℃) for 3 months, and then cultivated at 20℃ in the phytotron of Kyushu University for 5 months (Fig. 1-C, D).

Quantitative analysis of aconitine-type alkaloids

Mesaconitine, aconitine and hypaconitine contained in microtubers were analyzed by HPLC (Table 3) as previously reported (*10*) with the following modification. An HPLC system (Toso, Tokyo, Japan) composed of a CCPM pump and a UV-8000 absorbance detector equipped with a TSK-gel ODS-120A (4.6×250 mm) was used. For separation of aconitine-type alkaloids, the column was eluted with 20% of tetrahydrofuran containing 50 mM of phosphoric acid at a flow rate of 0.4 ml/min monitoring by absorption at 254 nm.

RESULTS AND DISCUSSION

In order to determine the effect of IAA on the transplantation of plantlets to soil, clonally propagated shoots were cultured on the MS medium supplemented with varing concentrations of IAA at 20℃ for 4 weeks as reported previously (*7*), and cultivated at 20℃ for 8 weeks individually (Table 1). Perfect establishments were obtained on plantlets precultured on the MS medium

Table 1. Effects of preculture in IAA containing MS medium on transplantaion of propagated plantlets of *Aconitum carmichaelii* to soil.

IAA (mg/l)	Establishment ratio (%)*	Av. No. of microtuber	Av. fresh weight (g)
0	100	1.5	0.94
0.5	100	2.1	2.16
1	100	2.2	1.72
2	80	2.0	1.40

Data scored after 8 week-cultivation at 20 ± 1℃.
*Ten clonally propagated plantlets were transplanted to soil.

supplemented with 0.5 and 1 mg/l IAA and without IAA. Culturing on the MS medium supplemented with 2 mg/l IAA weakly decreased the ratio of establishment. Interestingly the formation of microtubers occurred around the bottom of stem during 8-week-cultivation as indicated in Fig. 1-A. All of plantlets rooted on the MS medium supplemented with 0.5 mg/l of IAA produced approximately 2 microtubers, and 67% on that of 1 mg/l of IAA. From these results it became evident that the addition of 0.5 mg/l IAA enhanced the transplantation of plantlets and the formation of microtuber.

Previously we investigated the effect of cultivation temperature on growth of clonally propagated plants resulting that cultivation temperature affected the growth of the plant (*9*). The cultivation at 20℃ promoted the growth resulting in the highest fresh weight and microtuber formation (data not shown). The higher temperature than 20℃ inhibited the microtuber formation as previously reported (*9*).

Simple and rapid methodology for transplantation of regenerated plantlets is needed for the practical cultivation. Therefore, the regenerated plantlets were attempted to culture under the different temperature conditions in the growth cabinet for 6 weeks as indicated in Table 2. Although culturing at 20℃ enhanced the formation of microtuber as discussed above in the cultivation system, 3 microtubers per plantlet were induced at 15℃ under the dark condition (Fig. 1-B). Almost the same result as indicated in Table 2, was obtained by culturing at 20℃ resulting in 2.3 microtubers per plantlet. It is clear that the higher temparature than 20℃ inhibits the microtuber formation as the same with that of in the cultivation system (data not shown).

Table 3 shows the concentrations of aconitine-type alkaloids in microtubers. The contents of mesaconitine and hypaconitine were higher at higher temperature. This tendency is the same with the previous report (*10*) that analyzed the aconitine-type alkaloid contents after two year cultivation of microtubers in phytotron, although the concentrations of individual alkaloid shown in Table 3 were lower than that in the tubers of cultivated plant.

Finally the transplantation of microtuber to soil was carried out. When the microtubers propagated and divided were transplanted to vermiculite after stored in a refregerator (4℃) for 3 months and cultivated at 20℃, germination and

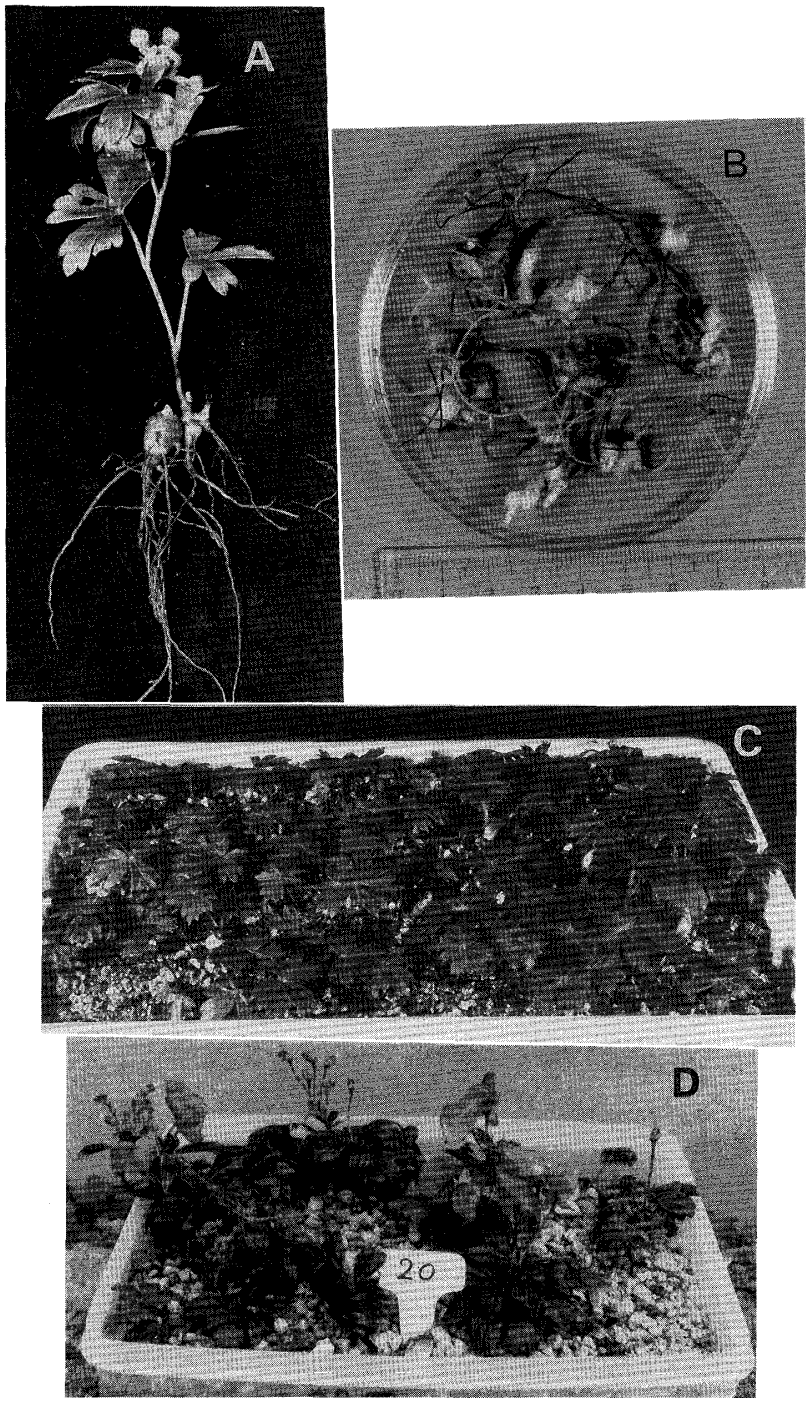

Fig. 1 A: Microtuber formation of rooted plantlets of *A. charmichaelii* under cultivation at 20℃ for 8 weeks. Clonally propagated shoots were cultured on the MS medium supplemented with 0.5 mg/1 IAA at 20℃ for 6 weeks to form roots. B: Microtuber formation of *A. charmichaelii* by culturing under the dark at 15℃ for 6 weeks. C: Germination of microtubers cultivated at 20℃. D: Cultivation of microtubers in phytotron at 20℃ for 5 months.

Table 2 Effects of culture temperature on microtuber formation of clonally propagated plantlets of *Aconitum carmichaelii.*

Temperature (℃)	Av. No. of* microtuber	Av. fresh weight of microtuber (mg)	Av. dry weight of microtuber (mg)
10	0.25	565.0	116.9
15	3.00	708.0	151.8
20	0.25	441.0	97.8

Data scored after 6 week-culturing under dark condition.
*Ten clonally propagated shoots were cultured.

Table 3 Effects of culturing temperature on aconitine-type alkaloid concentration of *Aconitum carmichaelii* Debx. microtubers.

Culturing condition (℃)	Average* μg/mg dry weight (c. v. %)**		
	Aconitine	Mesaconitine	Hypaconitine
10	0.0463±0.0020*** (4.3)	0.4690+0.0490 (10.4)	0.3000±0.0487 (16.3)
15	0.1350.±0.0102 (7.4)	0.6990±0.0076 (1.1)	0.3070±0.0167 (5.5)
20	0.0866±0.0061 (6.9)	1.0900±0.0413 (3.8)	0.8160±0.0713 (8.7)

*Microtubers produced as indicated in Table 2 were nalayzed.
**Coefficient variation.
***Standard error.

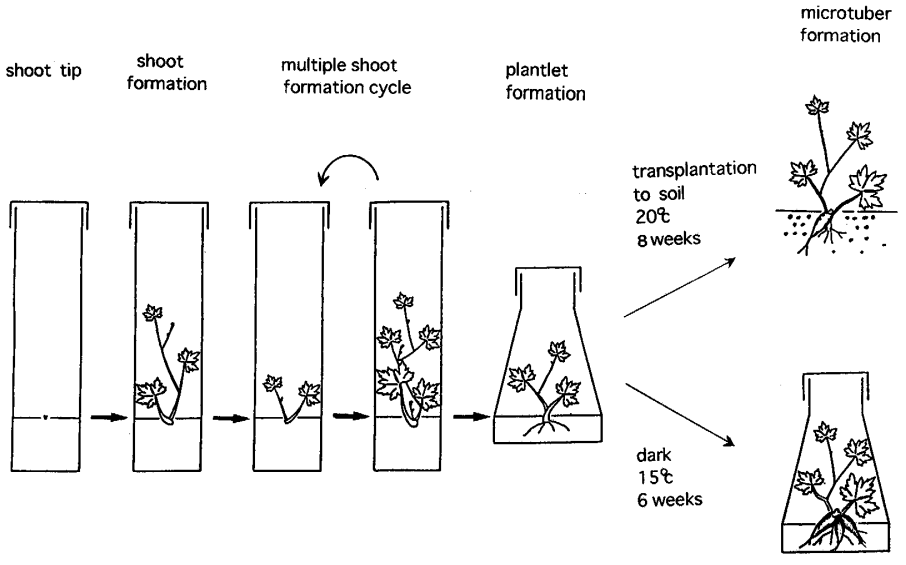

Fig. 2 Scheme of micropropagation and microtuber formation of *A. charmichaelii.*

establishment of transplantation were perfect (Fig. 1-C). Figure 1-D shows the plants grown from microtubers after 5-month cultivation period in the phytotron indicating some flowering occurred.

The preliminary cultivating experiment of microtubers propagated needed 2 years for reaching to adalt plants and untill all plants flowered (data not shown). As discussed in the previous paper, 1.2×10^7 clonal plants are obtained from a single shoot in a year (*7*). Therefore, 3.6×10^7 clonal microtubers are theoretically available from a single shoot in a year by culturing for more 6 weeks.

This is possibly the first report when microtubering and its application is described. Newly established propagation system for *A. carmichaelii* was indicated in Fig. 2. The approach described here may make possible the facile quick clonal propagation of this plant, suitable for the formation of pathogen free plantlets, and open the pratical cultivation of this medicinal and ornamental plants.

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