RELATIONSHIP BETWEEN THE CONTENT OF ACONITINE-TYPE ALKALOIDS AND GROWTH ENVIRONMENTS IN CLONALLY PROPAGATED ACONITUM CARMICHAELII DEBX

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出版情報：BIOTRONICS. 22, pp.87-93, 1993-12. Biotron Institute, Kyushu University
バージョン：
権利関係：

BIOTRONICS **22**, 87–93, 1993

RELATIONSHIP BETWEEN THE CONTENT OF ACONITINE-TYPE ALKALOIDS AND GROWTH ENVIRONMENTS IN CLONALLY PROPAGATED *ACONITUM CARMICHAELII* DEBX.

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(Received Aug. 16, 1993 ; Accepted Sept. 10, 1993)

SHOYAMA Y., YAMADA Y. and NISHIOKA I. *Relationship between the content of aconitine-type alkaloids and growth environments in clonally propagated* Aconitum carmichaelii *Debx.* BIOTRONICS 22, 87–93, 1993. The homogeneity of a population of clonally propagated *Aconitum carmichaelii* Debx. was confirmed by quantitative analysis of aconitine-type alkaloids by HPLC. Quantitative fluctuation of aconitine-type alkaloids occurs in relation to the harvest time of tubers. Cultivation temperature greatly affected the growth of the plant. The number of leaves and flowers increased at higher temperature, whereas lower temperature promoted tuber production. Plant height was proportional to the cultivation temperature, and the tuber weight was inversely proportional to it. The temperature clearly affected the production of aconitine-type alkaloids, demonstrating that the contents of mesaconitine and aconitine were higher at 25℃ than at 15℃. It is shown that quantitative fluctuation of aconitine-type alkaloids occurs in relation to the harvest time of tubers.

Key Words : *Aconitum carmichaelii* ; Ranunculaceae ; clonal propagation ; aconitine-type alkaloids ; cultivation ; phytotron.

INTRODUCTION

Aconitum carmichaelii Debx., is a perennial herb of the family Ranunculaceae, indigenous to China, of which the tubers have been 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. To decrease the toxicity of the crude drug, steam treatment (processing) has generally been carried out. During the treatment, a part of the individual aconitine-type alkaloids changes to the corresponding benzoylacontine-type alkaloids by deacetylation (*1*), to lipoaconitine-type alkaloids by transesterification (*2*), or to pyraconitine-type alkaloids (*3*) as indicated in Fig. 1, whose toxicity decreases, while the pharmacological activities increase. However, it is well-know that significant differences in the type and quantity of alkaloids have been observed depending

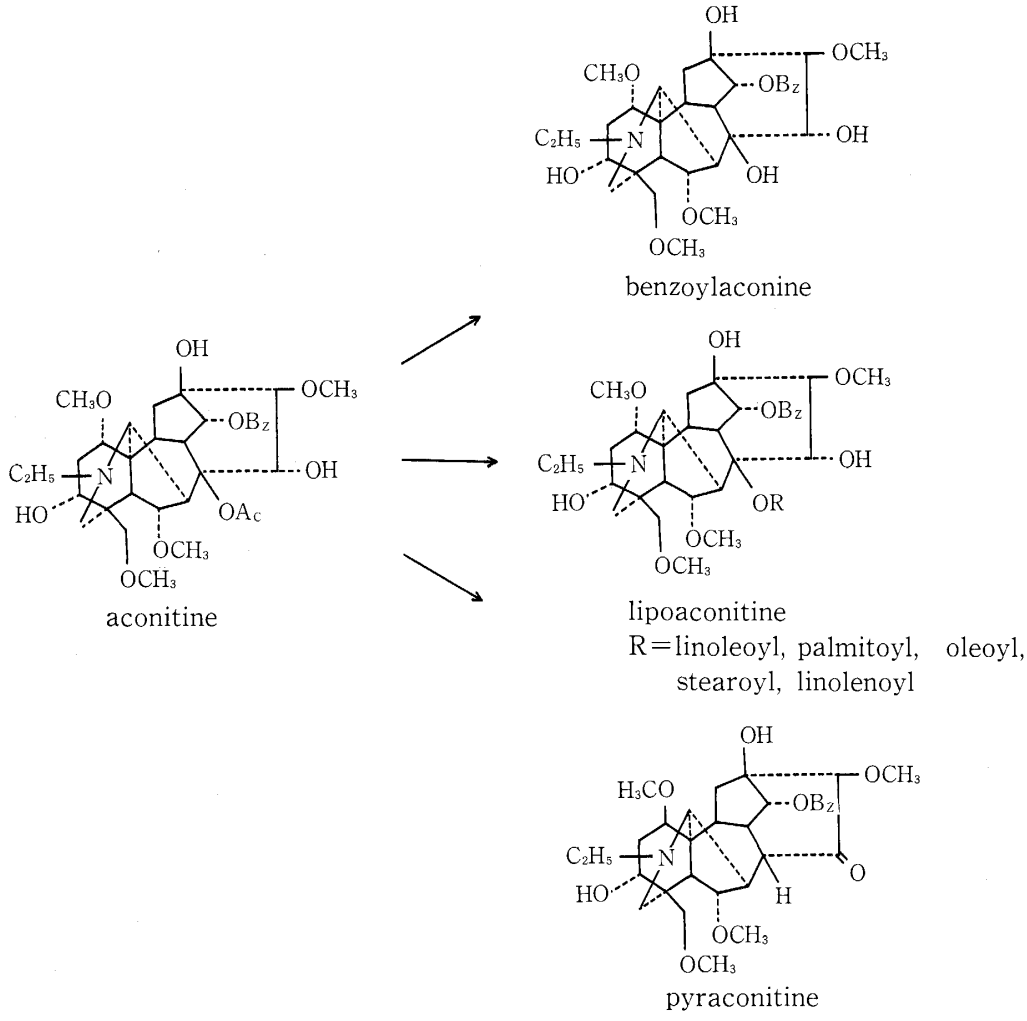

Fig. 1 Chemical transformation of aconitine-type alkaloids by processing.

on the place of growth (*4*, *5*) and the season (*6*, *7*). Therefore, in order to breed a homogeneous strain of *A. carmichaelii* Debx. with respect to the quality and quantity of aconitine-type alkaloids, we have already reported the clonal micropropagation of the plant by shoot tip culture as shown in Fig. 2; the subsequent restoration of adult plants and short-term-cultivation for the analysis of alkaloids (*8*), and also the micropropagation procedure by somatic embryogenesis via anther culture (*9*).

In this communication, we describe the correlation of aconitine-type alkaloids in clonally propagated plants and their growth temperatures.

MATERIAL AND METHODS

Plant material

Clonal *A. carmichaelii* Debx. plants were produced by shoot tip culture as shown in Fig. 2 (8). The microtubers were stored at 4℃ for 175 days until

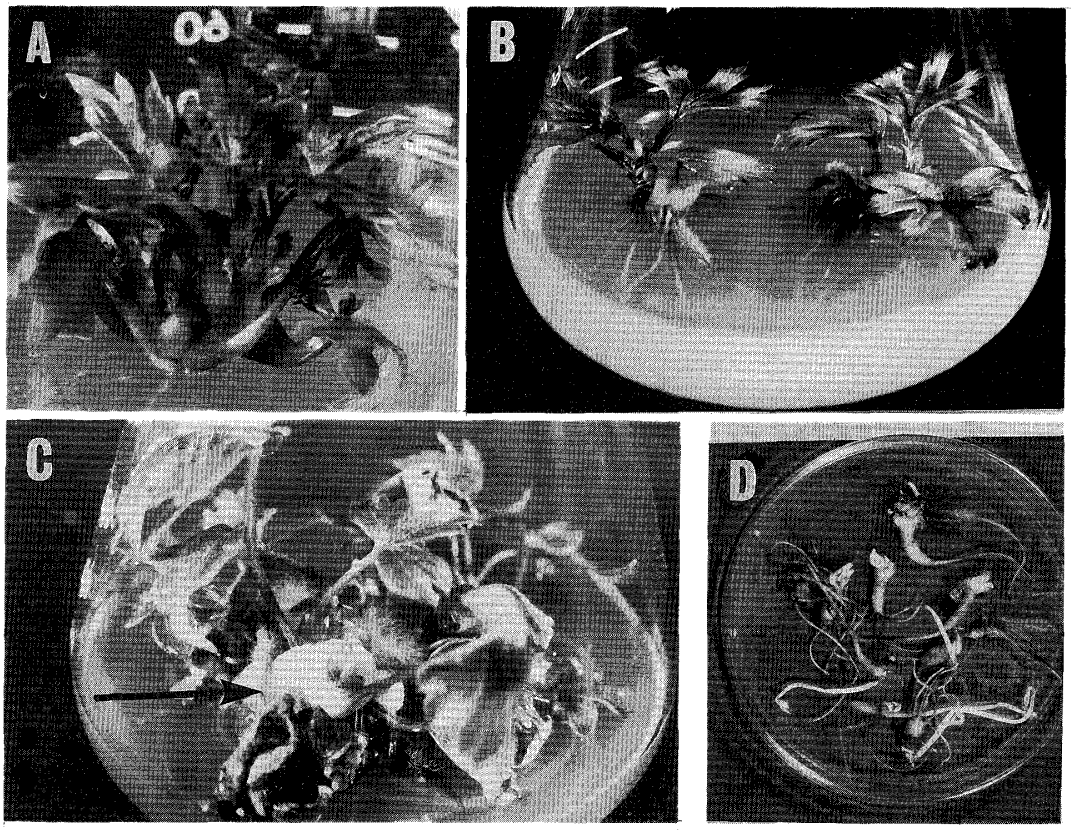

Fig. 2 Clonal propagation system of *Aconitum charmichaelii* Debx. by tip tissue culture.
A : Multiple shoot formation on MS medium supplemented with 5 mg/1 BAP.
B : Root formation on MS medium supplemented with IAA 0.5 mg/1.
C : Microtuber formation from rooted plantlet on MS medium supplemented with IAA 0.5 mg/1 under dark during 6-week-culture. Arrow shows microtuber formed.
D : Stored microtubers.

planting.

Cultivation in the phytotron and harvest

The stored 24 tubers were cultivated in a pot (3,780 cm^2) containing the mixture of clay and compost (3:1) in the phytotron of Kyushu University from May 8 to November 22 in 1989, and from May 6 to October 12, 1990. Hyponex dissolved in H_2O (1000 times) was applied once a week. A half of the plants were harvested on November 22, 1989 and the remainder were harvested on October 12, 1990.

To investigate the seasonal fluctuation of aconitine-type alkaloids the stored microtubers were transplanted during the month of May to the herbal garden, Faculty of Pharmaceutical Sciences, Kyushu University in 1987, and cultivated until February 1989.

Quantitative analysis of aconitine-type alkaloids

Aconitine-, hypaconitine- and mesaconitine-contents were analyzed by HPLC as previously described (*5*) using Gasukurokogyo Model 576 HPLC.

RESULTS AND DISCUSSION

Hikino et al. (*6*) and Yoshikawa et al. (*7*) presented the seasonal fluctuation of aconitine-type alkaloid contents in *A. carmichaelii*. However, the attempts were carried out using traditionally cultivated strains as a group. We have investigated here the seasonal fluctuation of the content using only one plant. The results are given in Fig. 3. The contents of mesaconitine, aconitine and hypaconitine were lowest in September and then gradually increased. The highest content was found in February. Furthermore, the aconitine-type alkaloid production was not related to the size of the daughter tubers in one plant (data not shown). From these results it is evident that quantitative fluctuation of aconitine-type alkaloids depends on the harvesting time of tubers as previously reported (*6, 7*).

There are several other contributing factors, such as growth temperature, soil fertility, humidity, light and so on, which affect the marked fluctuation in the content of aconitine-type alkaloid. It seems that the most important factor is temperature. Therefore, the clonally propagated plants were cultivated in the phytotron at 15, 20, 25 and 30℃ for 2 years. The results are Indicated in Table 1 and Fig. 4.

All plants died at 30℃ after a 2-month-cultivation. Therefore, the temperature lower than 25℃ is best for the cultivation of the plant.

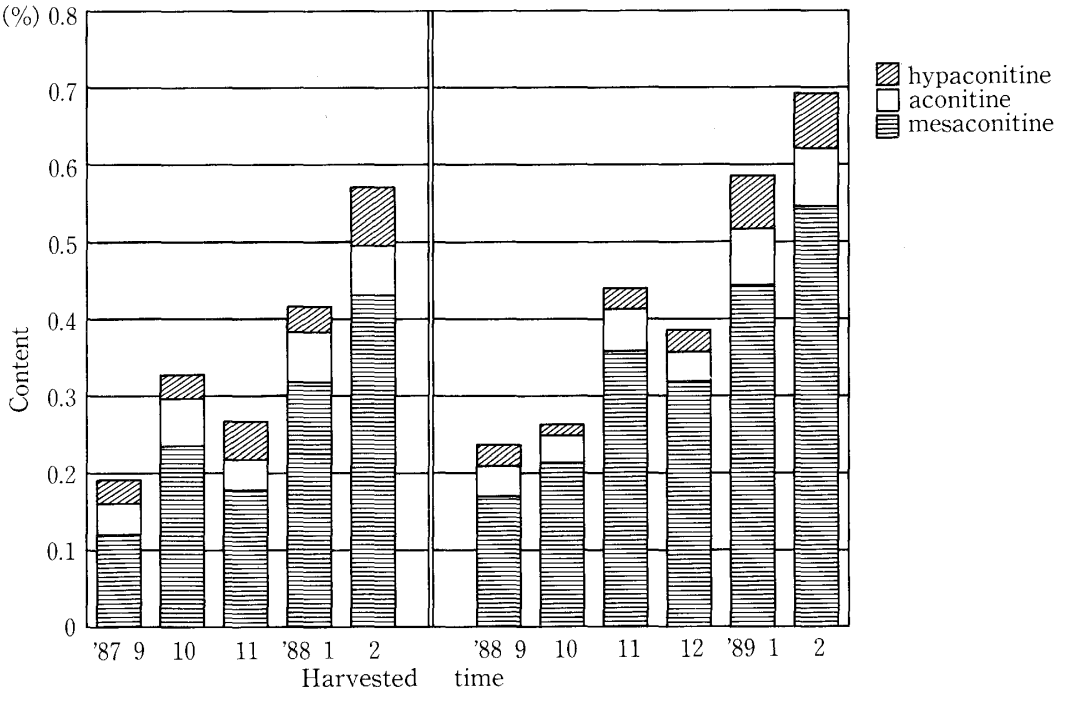

Fig. 3 Seasonal fluctuation of aconitine-type alkaloids.

Table 1. Effect of cultivation temperature on growth of clonally propagated plants of *Aconitum carmichaelii* using phytotron (First year).

Temperature (℃)	Survival ratio(%)	Av. No. of leaves	Av. No. of flowers	Av. No. of tuber	Av. fresh weight of tuber(g)
15	100	5.7	1.0	2.7	4.8
20	100	5.9	2.6	2.2	4.4
25	100	8.4	3.2	2.1	3.6
30	0	–	–	–	–

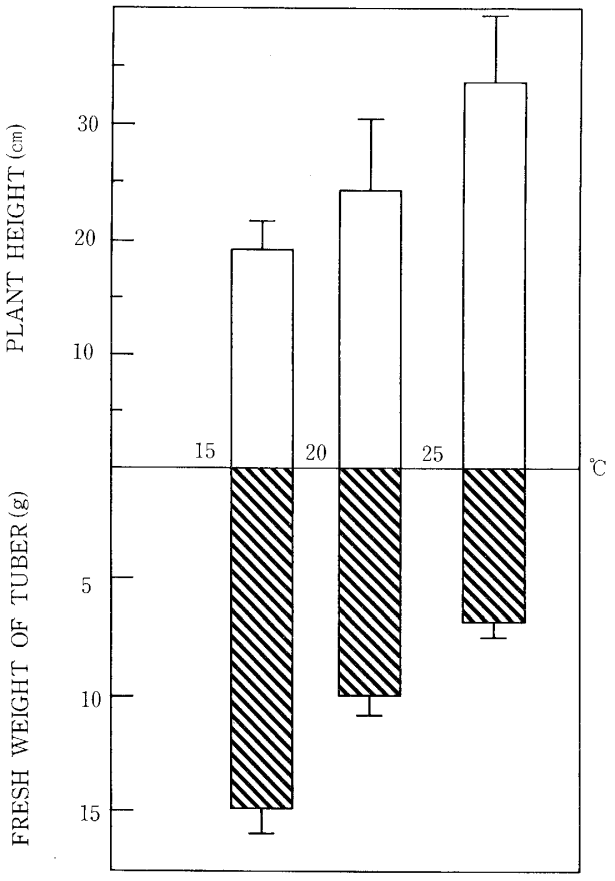

Fig. 4 Effect of cultivation temperature on growth of clonally propagated plants of *Aconitum carmichaelii* using phytotron (Second year).
Bar shows standard error.

Table 2. Effect of cultivation temperature on aconitine-type alkaloid contents of clonally propagated plants of *Aconitum carmichaelii* using phytotron (Second year).

Cultivation condition (℃)	N*	Average % of dry weight (c.v.%**)		
		Mesaconitine	Aconitine	Hypaconitine
15	10	0.158±0.016*** (9.8)	0.0145±0.0046 (31.7)	0.0499±0.0147 (29.4)
20	10	0.241±0.028 (11.5)	0.0372±0.0088 (23.6)	0.0580±0.0180 (31.0)
25	10	0.256±0.036 (14.0)	0.0605±0.0109 (18.0)	0.0326±0.0066 (20.2)
Field	70	0.127±0.032 (26.3)	0.0202±0.0087 (43.1)	0.0408±0.0170 (41.7)

* Sample number analyzed
** Coefficient variation
*** Standard error

Table 1 shows the effects of cultivation temperature on leaf-,flower- and tuber-number and tuber weight in the first year plants. Cultivation temperature greatly affected the growth of the plant. Increase of both leaf number and flower number occurred at higher temperatures. On the other hand, the lower temperatures stimulated propagation of tuber and also the increase of tuber weight.

Figure 4 shows the influence of cultivation temperature for the tuber weight and plant height in the second year plants. The plant height was proportional to the cultivation temperature. The plant height at 25℃ was almost twice as that at 15℃. The tuber weight was inversely proportional to the temperature, and the tuber weight at 25℃-cultivation was less than a half of that at 15℃, as indicated in Fig. 4. Moreover, the plants grown at 15℃ flowered after three-month-cultivation suggesting that the flowering time was shortened by cooler climate. From these results it became clear that the cooler climate is a more suitable for the root yield.

As shown in Table 2 the coefficient variation (c.v.) of individual aconitine-type alkaloids in the clonally propagated plants cultivated in the phytotron were smaller than those of parent plants cultivated in the field, suggesting that the clonal propagation by shoot tip culture provided the higher homogeneity of aconitine-type alkaloids. The contents of mesaconitine and aconitine were higher at higher temperature. The ratio of mesaconitine and aconitine (M/A) decreased as the temperature arose. This indicates that the growth temperature clearly affects the biosynthesis of aconitine-type alkaloid suggesting that clonally propagated plants of *A. carmichaelii* should be planted under the same condition in order to produce homogeneous crude drugs.

It has been believed that many pharmacologically active compounds contained in

the medicinal plants fluctuate depending on the growth environments. The relation is, however, still obscure due to many environmental factors. It becomes evident that at least the influence of temperature can be determined. Experiments regarding other medicinal plants have been investigated now using phytotron. Details will be presented elsewhere.

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