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RELATIONSHIP BETWEEN THE CONTENT OF FORSKOLIN AND GROWTH ENVIRONMENTS IN CLONALLY PROPAGATED *COLEUS FORSKOHLII* BRIQ.

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YANAGIHARA H., SAKATA R., SHOYAMA Y. and MURAKAMI H. *Relationship between the content of forskolin and growth environments in clonally propagated Coleus forskohlii Briq.* BIOTRONICS 24, 1-6, 1995. Temperature greatly affected the growth of *Coleus forskohlii* plant. The number of leaves and stems increased at higher temperature, whereas 20°C condition promoted flower production. The higher temperature stimulated tuberous root formation and its yield. Quantitative determination of forskolin content in the plants cultivated in different temperature was investigated by ELISA system using monoclonal antibody against forskolin. The highest forskolin content was found in the base of the tuberous root, and the content decreased towards the end of the organ. The temperature clearly affected the production of forskolin, demonstrating that the contents of forskolin was higher at 20°C than at 15 and 30°C.

Key words : *Coleus forskohlii*; Labiatae; clonal propagation; forskolin; ELISA; phytotron.

INTRODUCTION

Coleus forskohlii Briq., is a perennial herb of the family Labiatae. The plant originated from the Indian subcontinent, but has been distributed to Egypt, Arabia, Ethiopia, East Africa and Brazil (1). The tuberous roots have been used as an important folk medicine. Forskolin (Fig. 1), a labdane diterpenoid, was isolated from the tuberous roots (2) and found to be a typical activator of adenylate cyclase (3), leading to an increase of c-AMP, which affects heart action, blood and intraocular pressure. The production of forskolin is completely dependent on the commercial collection of wild and cultivated plants. It is reported that the natural resources have been exhausted in India (4). Therefore, in vitro culture systems of *C. forskohlii* have been investigated (5-7). We have already presented the production of monoclonal antibody (MAb) against forskolin and the sensitive assay system using ELISA (8, 9). Here we wish to

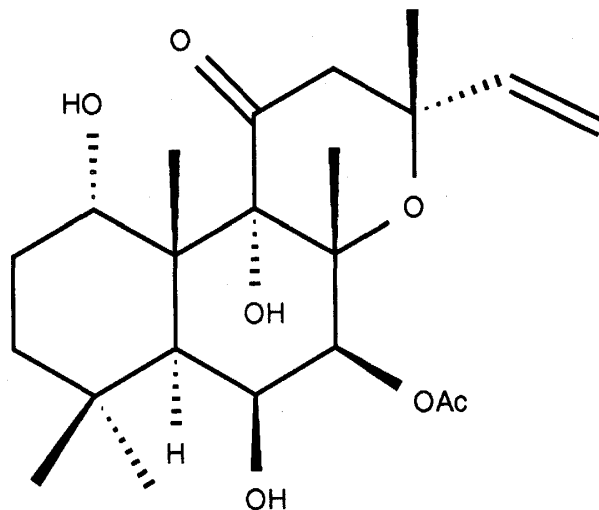


Fig. 1. Structure of forskolin.

communicate the correlation of forskolin contents and growth temperature of clonally propagated plants using phytotron.

MATERIALS AND METHODS

Cuttings of clonally propagated shoots

Shoot cuttings (1 cm in length) of *C. forskohlii* propagated by shoot tip culture as previously reported (6) were cultured in vermiculite with a plastic film cover in a greenhouse for 30 days until rooting.

Cultivation in phytotron and harvest

Plantlets rooted by cuttings were cultivated successively in vermiculite without plastic film cover for 180 days without fertilizer. Seven-month-old plants obtained by cuttings were transplanted in a mixture of clay and compost in May in 1993, and cultivated in the phytotron of Kyushu University at 15 ± 1 , 20 ± 1 and $30 \pm 1^\circ\text{C}$ under $70 \pm 5\%$ of humidity until September 28, 1993.

The number of leaves, stems, flowers and tuberous roots was counted immediately after harvest. Individual tuberous root was cut into pieces (approximately 1 cm in length). All samples were weighed after drying and extracted separately. The extracts were assayed after appropriate dilution for their forskolin content.

Quantitative analysis of forskolin

Quantitative analysis of forskolin was carried out as previously reported (9) in the following. Dried powder of tuberous roots (10 mg each) was extracted with acetone ($500 \mu\text{l}$) three times under sonication (15 min each) and then centrifuged (15,000 rpm, 3 min). The combined acetone solution was evaporated. The residue was redissolved in MeOH, diluted with phosphate buffer solution

(PBS) 16 times, and used for ELISA assay.

A 96 well-immunoplate which had been adsorbed by 100 μ l of 100 ng/ml forskolin-human serum albumin (HSA) was treated with 300 μ l of Gelatin-PBS. Fifty microliter of various concentration of forskolin was incubated for 1 hr with 50 μ l/ml of 1A9 (27.9 ng/ml) in other plate. After 1 hr 50 μ l of that mixture was displaced to F-HSA coated plate and incubated for 30 min. The plate was washed three times with PBS containing 0.05% Tween 20 (TPBS), and then the MAb was combined with 100 μ l of 1,000 times-diluted peroxidase-labeled anti-mouse IgG for 1 hr. After washing the plate three times with TPBS, 100 μ l of substrate solution was added to each well and incubated for 15 min. Absorbance at 405 nm was measured with a FAR 400 electrophotometer (SLT-LABINSTRUMENTS, Salzburg, Austria). All the reactions were carried out at 37°C. The forskolin contents are expressed in μ g per mg of dry weight of tuberous root.

RESULTS AND DISCUSSION

Clonal propagation of *C. forskohlii* has been established by Sen *et al.* (6). In this investigation we have improved the clonal multiplication system by cuttings as indicated in Fig. 2. In vitro propagated shoots were cut individually, and cultured in vermiculite directly without rooting stage. Perfect rootage was obtained (Fig. 2-B). Propagated plantlets grew normally and flowered from the end of July at 15°C, and the end of August at 20 and 30°C in the phytotron (Fig. 2-C). This phenomenon shows that *C. forskohlii* is a short-day plant. Figure 2-D shows the harvested tuberous roots of clonally propagated plantlets.

The relation between growth temperature and plant growth has been investigated as shown in Table 1. The number of leaves and stems per plant increased depending on higher temperature. The number of flowers, however, reached maximum at 20°C and then decreased at 30°C. Since *C. forskohlii* was propagated by seeds (1), the increase in flower number was predominant for propagation. Table 1 also shows that the number of tuberous roots increased at 20 and 30°C. The dry weight of tuberous root per plant was highest at 20°C, 4,630 mg, then 2,164 mg at 30°C. The total forskolin content per single plant was 9,230 and 58 mg at 20, 30 and 15°C, respectively. From these results the best temperature for the growth and forskolin production of *C. forskohlii* is 20°C.

Hayashi *et al.* (10) analyzed the content of glycyrrhizin in a growing stolon of *Glycyrrhiza glabra*, and found that the distribution of glycyrrhizin was greatly different from the stolon segments. Therefore, we surveyed the distribution of forskolin using the main tuberous root segment at 15, 20 and 30°C. The highest forskolin contents were found close to the tuberous root base and decreased towards the top of tuberous root (Table 2). This tendency was found at all temperature conditions. Fifteen °C apparently inhibited the production of forskolin together with the inhibition of tuberous root growth as previously discussed in Table 1. The highest forskolin content (7.87 μ g/mg dwt) was found in the base of tuberous root at 20°C. This content was approximately twice

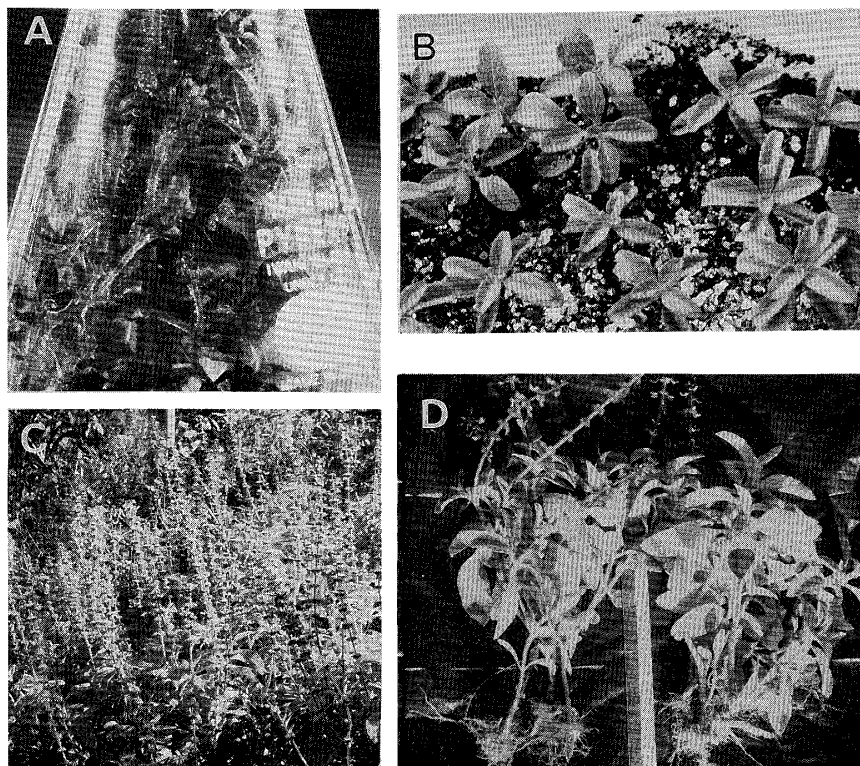


Fig. 2. Clonal propagation system of *Coleus forskohlii* by tip tissue culture.
 A: Multiple shoot formation on MS medium supplemented with 0.1 mg/l BAP.
 B: Cuttings of propagated shoots in vermiculite.
 C: Cultivation of clonally propagated *C. forskohlii* in phytotron.
 D: Harvested tuberous roots.

Table 1. Effect of cultivation temperature on growth and forskolin content of clonally propagated *Coleus forskohlii*¹⁾ in phytotron³⁾

Cultivation temp. (°C)	15±1	20±1	30±1
Number of leaves/plant	21	30	78
Number of stems/plant	5	8	13
Number of flowers/plant	5	24	8
Number of tuberous roots/plant ²⁾	6	14	14
Dry weigh of tuberous root/plant (mg)	524	4,630	2,164
Forskolin content/plant (mg)	9	230	58

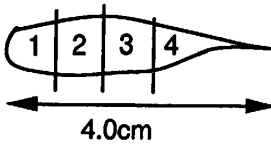
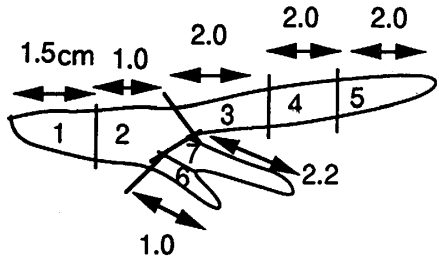
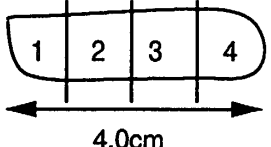
1) Single clonally propagated plant was used.

2) Standardized shape of major tuberous root used for analysis was indicated in Table 2.

3) Relative humidity was 70±5%.

compared to that of 30°C, and nearly three times higher than that of 15°C. The forskolin content of all other tuberous root segments at 20°C was higher than those of 15 and 30°C. This phenomenon has a good agreement with the result

Table 2. Distribution pattern of forskolin in different tuberous root segment in clonally propagated *Coleus forskohlii*¹⁾

Temp.	Tuberous root section ²⁾	Av. contents ($\mu\text{g}/\text{mg dwt}$) ³⁾	Shape of tuberous root ⁴⁾
15 \pm 1 $^{\circ}\text{C}$	1	2.83 \pm 0.28	
	2	1.36 \pm 0.39	
	3	1.24 \pm 0.23	
	4	1.15 \pm 0.16	
20 \pm 1 $^{\circ}\text{C}$	1	7.87 \pm 0.35	
	2	6.24 \pm 0.49	
	3	6.55 \pm 1.25	
	4	5.73 \pm 0.61	
	5	4.49 \pm 0.49	
	6	5.49 \pm 0.34	
	7	5.08 \pm 0.65	
30 \pm 1 $^{\circ}\text{C}$	1	3.83 \pm 0.38	
	2	3.57 \pm 0.24	
	3	2.99 \pm 0.30	
	4	1.92 \pm 0.42	

- 1) Single clonally propagated plant as indicated in Table 1 was used for analysis.
- 2) The main tuberous roots cultivated at 15 and 30 $^{\circ}\text{C}$ were sectioned into pieces (10 mm each), and those at 20 $^{\circ}\text{C}$ were cut into pieces as indicated in the shape, respectively. Section No.1 shows the base of tuberous root.
- 3) A single plant was tested individually, and the content was expressed by the average of 3 measurements.
- 4) Standardized tuberous root was selected and used for analysis.

discussed already that the optimum temperature condition for the growth and forskolin production is 20 $^{\circ}\text{C}$.

Previously, we found the correlation between the growth temperature and aconitine-type alkaloid contents in *Aconitum carmichaeli* (11). Similarly in this investigation the authors clarified a substantial growth of *C. forskohlii* and accumulation of forskolin depending on growth temperature condition, moreover a clear distribution pattern of forskolin in tuberous root segment. Therefore, it becomes evident that the growth conditions should be controlled when compares the forskolin content in individual *C. forskohlii* plants.

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