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Production of (+)-Catechin in Root and Cell Suspension Cultures of *Rheum palmatum* L.

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Root and cell suspension cultures of *Rheum palmatum* were established and analyzed for the production of (+)-catechin. The effects of media and growth regulators on the growth and (+)-catechin formation in the root and cell cultures were also investigated. The content of (+)-catechin in the cells, cultured in MS liquid medium containing 1 mg/*l* 2, 4-D, reached the level of 0.38% (dry weight, dw) at week 7 of the culture. In contrast, the root cultured in MS liquid medium containing 2 mg/*l* NAA showed the highest level of (+)-catechin (0.24%, dw) at week 1 of the culture.

Rhubarb ('Daio' in Japanese), the rhizome and root of *Rheum* spp. (Polygonaceae), is one of the most important traditional Chinese medicines used in combination with other crude drugs for blood-stasis syndrome, hypertension, renal disorder, diarrhea, and so on. Recently, some new biological activities such as psychotropic,[1] improvement of nitrogen metabolism,[2-5] and inhibition of angiotensin-converting enzyme[6] were discovered and proved to be originated from its tannin constituents (rhatannins and RG-tannin). In spite of many phytochemical, biochemical, and pharmacological examinations, there have been few reports on tissue culture products (anthraquinones[7] and sennosides[8]) of rhubarb plants. We have succeeded in the establishment of root and cell suspension cultures of *R. palmatum* and *in vitro* formation of (+)-catechin (**1**), which is the structural element of rhatannins and RG-tannin. We also determined the effects of media and growth regulators on the growth and production of **1** in the root and cell cultures by HPLC analysis.

Material and Methods

Plant material and induction of calli and adventitious roots. The petioles, cut from *R. palmatum* plants cultivated in the field at Tsukuba Medicinal Plant Research Station, were surface-sterilized with 3% sodium hypochlorite with Tween 20 (1 drop/50 ml) and then washed with sterilized water three times. The petiole segments (2 mm thickness) were aseptically inoculated on Murashige-Skoog[9] (MS) solid media (containing 2 g/*l* Gelrite) with various concentrations of 2, 4-D, IAA, or NAA and/or BA (**Table 1**) in the dark at 25℃. The formation rates of calli and adventitious roots are shown in **Table 1**.

Cell suspension culture. The small pieces of the calli which formed on the segments cultured on MS solid medium containing 1 mg/*l* 2, 4-D were transferred to the same medium and subcultured every two months. The calli were also inoculated to MS liquid medium (50 ml/100 ml Erlenmeyer flask) containing 1 mg/*l* 2, 4-D to establish the cell suspension culture. The cultures were maintained in the dark at 25℃ on a rotary shaker (100 rmp) for over one year.

To determine the growth of the cells and the production of **1**, *ca.* 1.3 g (fresh weight, fw) of the cells were inoculated in the same medium and cultured for 1-8 weeks with the same conditions men-

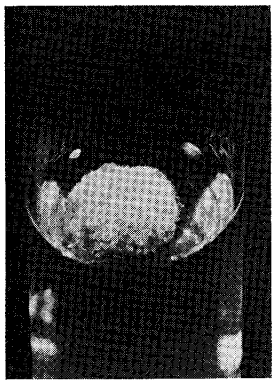

Fig. 1. Induction of the callus on the petiole segment cultured on MS medium containing 1 mg/*l* 2, 4-D.

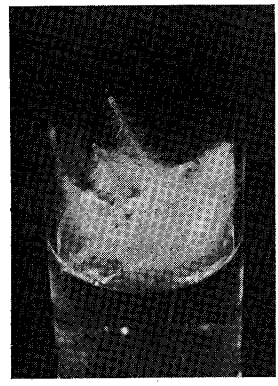

Fig. 2. Induction of the adventitious root on the petiole segment cultured on MS medium containing 0.5 mg/*l* NAA.

tioned above.

Root culture. The roots induced on the segments cultured with 0.5 or 2 mg/*l* NAA were cut off and transferred to MS liquid medium (50 ml/100 ml flask) containing 2 mg/*l* NAA and maintained for over one year. The culture conditions were the same as described above.

The roots (*ca.* 100 mg, fw) were inoculated to three different media [MS medium containing 2 mg/*l* NAA; Gamborg B 5[10] (B 5) medium containing 0.5 mg/*l* NAA or 0.5 mg/*l* IAA (each 50 ml/100 ml flask)] and cultured for 1–8 weeks to investigate the growth of the roots and the production of **1**.

Isolation of **1** *from the adventitious roots.* The adventitious roots, cultured in MS liquid medium (containing 2 mg/*l* NAA) for 4 weeks, were lyophilized (16.3 g, dw) and extracted with 80% aqueous acetone (150 ml×3). The extract, after concentration, was partitioned with AcOEt. The AcOEt layer, after evaporated to dryness, was chromatographed over Sephadex LH-20 (EtOH, 60% MeOH) to afford **1** (17 mg). **1** was identified as (+)-catechin by comparison of its physical and spectral data (PMR, $[\alpha]_D$, *etc.*) with those of the authentic sample.

HPLC analysis. Samples (*ca.* 50 mg, dw) were extracted with 80% acetone (4 ml) in a sonicator (20 min) at room temperature. Each extract, after filtration, was evaporated to dryness. The samples were dissolved with H_2O (3 ml) and partitioned with AcOEt (2 ml×1, 1 ml×3). The AcOEt solution, after evaporation, was dissolved with MeOH (150 μl), filtered by Millipore (0.45 μm), and injected (10 μl) to HPLC. HPLC conditions were as follows: column, Nucleosil 100-5 C_{18} (4.6 mm ID×250 mm); mobile phase, CH_3CN-0.05 M H_3PO_4 (15 : 85); column temp., 40℃; flow rate, 0.8 ml/min; detect, UV 280 (nm); t_R, 1 (8.00 min).

Results and Discussion

Induction of callus and adventitious root

When the segments of petioles were cultured on MS solid medium containing 0.1 or 1.0 mg/*l* 2, 4-D, large amounts of white calli were induced in all of the explants (**Fig. 1, Table 1**). The calli, subcultured on MS solid medium supplemented with 1 mg/*l* 2, 4-D, turned light brown with dark brown parts after week 4. This coloration was presumed to be caused by the complexation of the phenolic compounds in the calli and metal ion contained in the culture medium. NAA and IAA slightly stimulated the formation of the callus on the petiole segments. On the other hand, the addition of NAA (not combined with BA) showed a prominent effect on the induction of the adventitious root (**Fig. 2**). When the petiole explants were cultured with 0.5 or 2.0 mg/*l* NAA, 3 to 15 adventitious roots were induced on each segment with about 50% to 60% of frequency. 2, 4-D and IAA did not show the preferable effect on the formation of the adventitious root.

Table 1. Effects of the growth regulators on callus and root formation in petiole segment of *R. palmatum* cultured on MS solid medium.

Growth regulators (mg/*l*)				Callus (mg)[a]	Root (No.)[b]
2, 4-D	IAA	NAA	BA		
0. 1			0. 1	1600	0
0. 1				800	0
1. 0			0. 1	1600	0
1. 0				2000	0
		0. 5		320	10. 8
		2. 0		290	7. 0
		0. 5	0. 1	110	0
		2. 0	0. 1	430	0
	3. 0			170	0. 6
	3. 0		0. 1	550	0

Ten petioles were inoculated on each medium and cultured at 25℃ in the dark for 2 months.

[a] average fresh weight of calli per one petiole.

[b] average number of roots per one petiole.

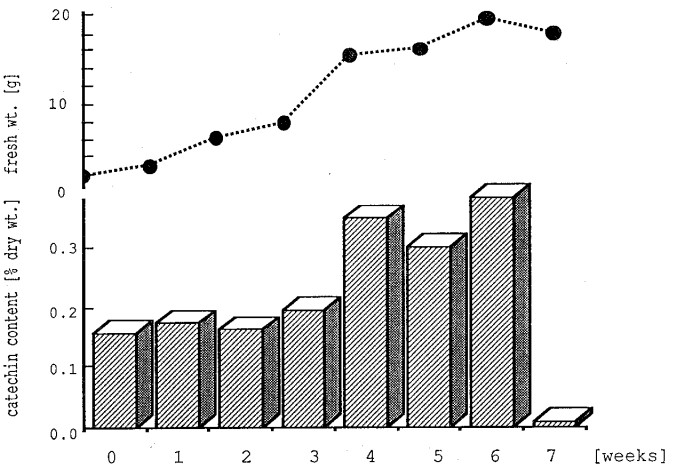

Fig. 3. Growth and catechin content in cells of *Rheum palmatum* cultured in MS liquid medium containing 2, 4-D (1 mg/*l*).

Cell suspension culture

The growth of the cells cultured in MS liquid medium containing 1 mg/*l* 2, 4-D increased from the beginning of the culture and after it reached the maximum level at week 6 it began to decrease slightly (**Fig. 3**).

The level of the content of **1** in the cells (**Fig. 3**) was almost at a plateau (*ca.* 0. 18%, dw) in the early stage of the culture (0-3 weeks), and when the cells reached the logarithmic phase of the growth (3 weeks) it began to increase. After it reached the highest level (0. 38%, 6 weeks), the content of **1** rapidly decreased and at the end of the culture period (7 weeks) it showed tolerably low content (0. 008 %). At week 7, the cells showed dark brownish coloration and some of them began to die. The sudden decrease of **1** at this stage was, therefore, presumed to be caused by its conversion, degradation, and/or complexation with the cell organ products in accordance with the destruction of the cells (the details were obscure).

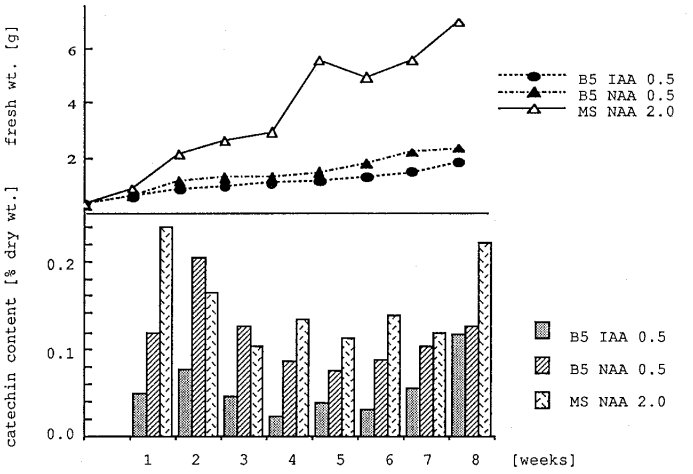

Fig. 4. Growth and catechin content in adventitious roots of *Rheum palmatum* cultured in MS (2 mg/*l* NAA) and B 5 (0.5 mg/*l* NAA, 0.5 mg/*l* IAA) liquid media.

Adventitious root culture

Among the three media tested, MS liquid medium supplemented with 2 mg/*l* NAA exhibited the most preferable effect on the growth of the adventitious roots (**Fig. 4**). The growth rate of the roots cultured in this medium was approximately three times larger than that of those cultured in B 5 medium containing 0.5 mg/*l* NAA or 0.5 mg/*l* IAA. In the B 5 media supplemented with 0.5 mg/*l* NAA or 0.5 mg/*l* IAA, there was not much difference in the effects on the growth of the roots.

On the other hand, the content of **1** in the roots cultured in B 5 liquid medium containing 0.5 mg/*l* NAA was *ca.* three times larger than that of the one cultured in B 5 medium supplemented with 0.5 mg/*l* IAA. The roots, cultured in MS liquid medium containing 2 mg/*l* NAA showed the highest level of the content of **1** for the most part of the culture period (1 week, 4–8 weeks). The maximum level of the content of **1** in the roots cultured in these three media was observed at the early stage of the culture (2 weeks). In the rapid growth of the roots cultured in MS liquid medium containing 2.0 mg/*l* NAA, the highest content of **1** in the roots was observed within the first week of the culture. The small increment of **1** at the end of the culture was different from the case of the cell suspension culture.

The contents of **1** in the root and cell suspension cultures were comparatively lower than those in the plants of *Rhubarb* spp. (*R. palmatum*—6.06%, dw; *R. officinale*—1.18%, dw).[11] But, taking into account the rapid growth of the cell and root cultures, these cultures seem to be useful for the production of **1**. The root and cell suspension cultures of *R. palmatum* may be able to produce high molecular condensed tannins (rhatannins and RG-tannin).

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References

1) Ueki, S., G. Nonaka, I. Nishioka and M. Fujiwara, 1985. J. Med. Pharm. Soc. for WAKAN-YAKU, **2**: 502-503.
2) Nagasawa, T., S. Shibutani, H. Oura, 1978. Yakugaku Zasshi, **98**: 1642-1650.
3) Nagasawa, T., S. Shibutani, H. Oura, 1980. Yakugaku Zasshi, **100**: 434-442.
4) Nagasawa, T., H. Oura, Y. Shoyama, I. Nishioka, 1980. Chem. Pharm. Bull., **28**: 1736-1739.
5) Shibutani, S., T. Nagasawa, H. Oura, G. Nonaka, I. Nishioka, 1983. Chem. Pharm. Bull., **31**: 2378-

2385.
6) Inokuchi, J., H. Okabe, T. Yamauchi, A. Nagamatsu, G. Nonaka, I. Nishioka, 1985. Chem. Pharm. Bull., **33**: 264-269.
7) Furuya, T., S. Ayabe, K. Noda, 1975. Phytochemistry, **14**: 1457.
8) Ohshima, Y., K. Takahashi and S. Shibata, 1988. Planta Med., **54**: 20-24.
9) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
10) Gamborg, O. L., R. A. Moller, K. Ojima, 1968. Exp. Cell. Res., **50**: 151-158.
11) Kashiwada, Y., G. Nonaka, I. Nishioka, 1989. Chem. Pharm. Bull., **37**: 999-1004.

≪和文要約≫

ダイオウ (*Rheum palmatum* L.) の細胞懸濁および根培養における (+)-catechin 生産

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ダイオウ (*Rheum palmatum* L.) の葉柄よりカルスおよび不定根を誘導し，それらの液体培養系を確立した．細胞懸濁培養では 1 mg/*l* 2,4-D 添加 MS 培地，不定根培養では 2 mg/*l* NAA 添加 MS 培地において，良好な生育と (+)-catechin 生産が認められた．