Pergamon

0031-9422(95)00849-7

Phytochemistry, Vol. 42, No. 1, pp. 69–72, 1996

0031-9422/96 $15.00 + 0.00

POLYACETYLENES IN HAIRY ROOTS OF *PLATYCODON GRANDIFLORUM*

JUN CHEUL AHN, BAIK HWANG, HIROMI TADA,* KANJI ISHIMARU,* KAZUO SASAKI† and KOICHIRO SHIMOMURA†‡

Department of Biology, Chonnam National University, 300 Yongbong-Dong, Kwangju 500–757, Korea; *Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, 1 Honjo, Saga 840, Japan; †Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, 1 Hachimandai, Tsukuba, Ibaraki 305, Japan

(*Received in revised form* 10 *October* 1995)

Key Word Index—*Platycodon grandiflorum*; Campanulaceae; Korean balloon flower; hairy root; *Agrobacterium rhizogenes*; polyacetylene; lobetyol; lobetyolin; lobetyolinin.

Abstract—Hairy roots of Korean balloon flower (*Platycodon grandiflorum*) were induced from root tissues infected with *Agrobacterium rhizogenes* ATCC 15834. Growth and polyacetylene (lobetyol, lobetyolin and lobetyolinin) production of 10 hairy roots clones cultured in 1/4 Gamborg B5 (B5) liquid medium were determined. One selected hairy root clone (D6) grew well in hormone-free B5 liquid medium and showed maximum content of polyacetylenes at week 6 for lobetyol (0.375% dry wt) and at week 7 for lobetyolin and lobetyolinin (3.030 and 0.206% dry wt, respectively) whose levels were much higher than those of the intact plant root (lobetyol: 0.019%; lobetyolin: 0.077% dry wt; lobetyolinin was not detected).

INTRODUCTION

Platycodon grandiflorum is a valued horticultural plant widely planted for its balloon-shaped flower. In Korea, the balloon flower is largely cultivated for the supply of edible roots. The root of the plant, containing saponins [1–4], has also been used as an expectorant for cough and bronchitis, a sedative and an analgesic in oriental medicine.

Recently, three new polyacetylenes (containing a conjugated diyne structure) were isolated from hairy root cultures of *Lobelia inflata* by Ishimaru *et al.* [5, 6] and named lobetyol (**1**), lobetyolin (**2**) and lobetyolinin (**3**). It was reported that the content of these polyacetylenes in hairy root culture of *L. inflata* was higher than that in the roots of the intact plants [7, 8]. In *L. sessilifolia* [6] and *P. grandiflorum* [9] this fact was also demonstrated. In addition, it has been reported that these polyacetylenes can be used as important chemotaxonomic markers in campanulaceous plants [9].

We describe the establishment of hairy root cultures from roots of *P. grandiflorum* purchased at a market in Korea. Selection of high polyacetylene-yielding hairy root clone and its optimal culture conditions for growth and polyacetylene production were investigated.

RESULTS AND DISCUSSION

For hairy root induction of *P. grandiflorum*, edible roots sold at a market in Korea were infected with *Agrobacterium rhizogenes* ATCC 15834. Tips of hairy roots induced on the root discs were individually excised and cultured on 1/4 Gamborg B5 (B5) [10] or 1/10 Murashige–Skoog (MS) [11] solid medium. Although some differences in growth pattern, such as growth rate, density of root hairs and branching ability on solid media, were observed, 10 clones (D1–10) showing rapid growth in several passages were selected and used in this study. In all selected clones, the integration of both TL- and TR-DNAs (from Ri plasmid in *Agrobacterium*) was confirmed by the detection of opines (agropine and mannopine) and polymerase chain reaction (PCR) analysis (data not shown).

To select the high polyacetylene-yielding clone, growth and polyacetylene content of 10 clones after culturing in 1/4 B5 liquid medium for 4 weeks were determined (Fig. 1). In these hairy root cultures, significant difference in growth (48.2 (D7)–102 (D3) mg dry wt per flask) and polyacetylene content (0.54% (D5)–2.3% (D4) dry wt, total amount of **1–3**) were observed. Among these clones, D6, which showed good growth (84.3 mg dry wt per flask) and polyacetylene production (2.0% dry wt of **1–3**), was selected for the following experiments.

For the determination of the optimal medium for the growth and polyacetylene production of D6, various liquid media (MS, B5, Woody Plant (WP) [12], half-strength of MS, B5, WP and quarter strength of MS, B5, WP) were tested. As shown in Fig. 2, B5 medium was best for growth (3.02 g, fresh wt per flask) and

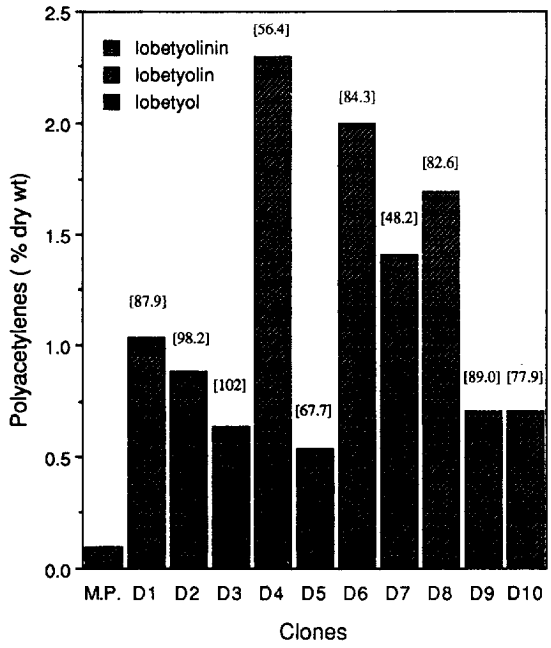
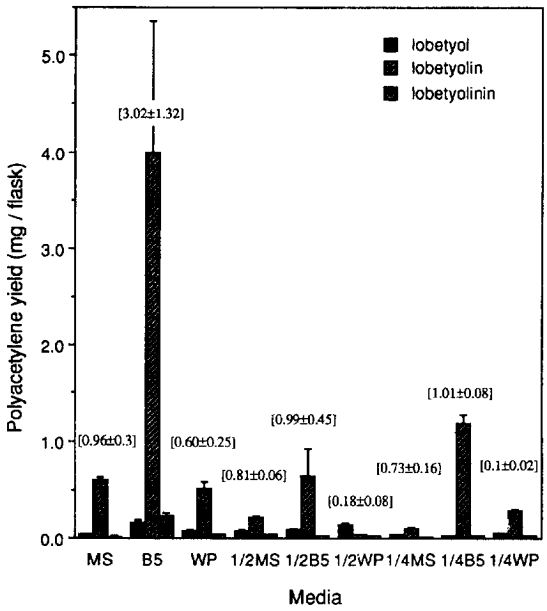
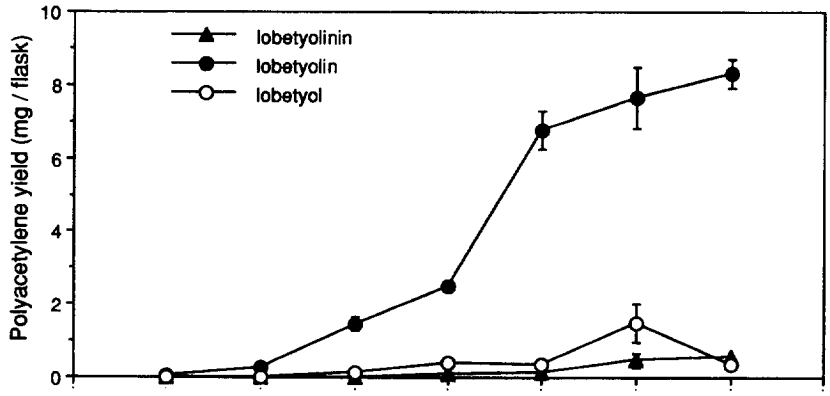


Fig. 1. Growth and polyacetylene production of *Platycodon grandiflorum* hairy root clones cultured in one-quarter strength B5 liquid medium for 4 weeks at 25° in the dark. Values in brackets show dry weight (mg 100 ml^{-1} flask). M.P., root of mother plant.

polyacetylene production of the clone D6 in B5 liquid medium was also investigated. Rapid growth started after week 2 and continued until week 6, when the highest root weight (fr. wt: 5.06 g; dry wt: 0.28 g per flask) was observed. The production of polyacetylenes (in particular **2**) roughly paralleled the root growth, showing a rapid increment after week 2 and reached the highest yield at week 6 (**1**: 1.46 mg per flask) and at week 7 for **2** and **3** (**2**: 8.29 mg; **3**: 0.5 mg per flask) (Fig. 3). The content of these polyacetylenes (% dry wt) in clone D6 was *ca* 40 times higher than that in parent plants (**1**: 0.02%; **2**: 0.08%; **3**: not detected) which had been used for hairy root induction.

The maximum polyacetylene yield of hairy root (D6) induced from the Korean balloon flower was almost five times larger than that of the hairy roots (induced by *A. rhizogenes* MAFF 03-01724) from Japanese plants [9]. This result demonstrated the importance of the selection of hairy root clones (together with *Agrobacterium* strains used for hairy root induction) and the





Fresh weight (g per 100 ml flask ± standard error)	0.05±0.01	0.25±0.08	1.70±0.44	1.99±0.23	3.54±0.63	5.06±0.94	4.51±0.22
Dry weight (g per 100 ml flask ± standard error)	0.004±0.001	0.016±0.005	0.114±0.023	0.142±0.009	0.209±0.021	0.281±0.031	0.271±0.009
Weeks	1	2	3	4	5	6	7

Fig. 3. Polyacetylene production in *Platycodon grandiflorum* hairy root clone D6 cultured in B5 liquid medium for 7 weeks at 25° in the dark. Bars represent standard errors.

determination of optimal culture conditions (medium, culture periods, etc.) for the high production of useful secondary metabolites (polyacetylene, etc.) in this species. To clarify the differences between Korean and Japanese types of *P. grandiflorum*, cytological and DNA analyses may be required with regard to taxonomy.

EXPERIMENTAL

Establishment of hairy root cultures. Platycodon grandiflorum A. DC roots were purchased at a market in Kwangju, Korea. They were sterilized with a NaOCl soln (available chlorite concn 3%) containing Tween 20 for 15 min, followed by washing ×3 with distilled H_2O. Roots were then aseptically cut into *ca* 1 cm thick sections. These disinfected root discs were used for *Agrobacterium* infection. *A. rhizogenes* ATCC 15834 subcultured on YEB [13] agar medium was inoculated by a needle onto the upper surface of the root discs. About 3–4 weeks after infection, several hairy roots appeared at the inoculated sites. The hairy roots were excised and placed on hormone-free 1/4 strength macro elements B5 medium supplemented with 0.75% agar and 0.5 mg ml^{-1} Claforan, an antibiotic for the elimination of bacteria. Axenic hairy roots were transferred to hormone-free 1/4 B5 liquid medium or 1/10 MS liquid medium (1/10 macro elements of MS basal medium, 30 ml per 100 ml flask) and cultured at 25° in the dark (100 rpm, on a rotary shaker). Ten clones (D1–10) were selected and growth and polyacetylene production were examined. Transformation of these hairy roots was confirmed by the detection of opines (agropine and mannopine) using paper electrophoresis and by confirming the insertion of TL and TR-DNA into the plant genome DNA using PCR with *rol* A-1, *rol* B-2 (for TL) and AGS-1, AGS-2 (for TR) primers. Hairy roots were cultured at 25° in the dark (100 rpm, on a rotary shaker). Hairy roots were harvested after 4 weeks of culture and the growth (fresh and dry wt) and polyacetylene content examined.

Medium for optimal growth and high polyacetylene production of clone D6. Hairy root clone D6 (*ca* 100 mg fr. wt) was inoculated into three different hormone-free liquid media, MS, B5, WP containing 1/4, 1/2 and full-strength macro elements of basal media, and cultured at 25° in the dark (100 rpm, on a rotary shaker). Hairy roots were harvested after 4 weeks of culture and the growth (fresh and dry wt) and polyacetylene content/determined.

Time-course of growth and polyacetylene production of clone D6 in B5 medium. Hairy roots (*ca* 100 mg) were cultured in hormone-free B5 liquid medium (30 ml per 100 ml flask) at 25° in the dark (100 rpm, on a rotary shaker). Hairy roots were harvested weekly (1–7 weeks) and fresh wt, dry wt and polyacetylene content/determined.

Analysis of polyacetylenes. Sample prepn and HPLC conditions for the quantification of polyacetylenes (**1**–**3**) were as described in ref. [8].

Acknowledgements—The authors thank Dr L. Jouanin for providing pLJ 1 and pLJ 85, Mr Hideki Matsumoto for PCR analysis and Ms W. S. C. Shu for critical reading of our manuscript. This work was supported in part by the Korea Science and Engineering Foundation.

REFERENCES

1. Tada, A., Kaneiwa, Y., Shoji, J. and Shibata, S. (1975) *Chem. Pharm. Bull.* **23**, 2965.
2. Ishii, H., Tori, K., Tozyo, T. and Yoshimura, Y.

(1978) *Chem. Pharm. Bull.* **26**, 668.
5. Ishimaru, K., Yonemitsu, H. and Shimomura, K. (1991) *Phytochemistry* **30**, 2255.
6. Ishimaru, K., Arakawa, H., Yamanaka, M. and Shimomura, K. (1994) *Phytochemistry* **35**, 365.
7. Ishimaru, K., Sadoshima, S., Neera, S., Koyama, K., Takahashi, K. and Shimomura, K. (1992) *Phytochemistry* **31**, 1577.
8. Ishimaru, K., Arakawa, H. Sadoshima, S. and Yamaguchi, Y. (1993) *Plant Tissue Cult. Letters* **10**, 191.
9. Tada, H., Shimomura, K. and Ishimaru, K. (1995) *J. Plant Physiol.* **145**, 7.
10. Gamborg, O. L., Miller, R. A., Ojima, K. (1968) *Exp. Cell. Res.* **50**, 151.
11. Murashige, T. and Skoog, F. (1962) *Physiol. Plant.* **15**, 473.
12. Lloyd, G. B. and McCown, B. H. (1980) *Int. Plant. Prop. Soc.* **30**, 421.
13. Vervliet, G., Holsters, M., Teuchy, H., van Montague, M. and Schell, J. (1975) *J. Gen. Virol.* **26**, 33.