J. Plant Biol. 38(4) : 337~341 (1995)

Induction and Culture of High Polyacetylene-Yielding Hairy Roots in Balloon Flower (*Platycodon grandiflorum*)

Ahn, Jun Cheul, Sang Seop So[1], Koichiro Shimomura[2] and Baik Hwang*

Department of Biology and Hormone Research Center, Chonnam National University, Kwangju 500-757, Korea;

[1]*Department of Biology Education, Chonbuk National University, Chonju 561-756, Korea; and*

[2]*Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, Tsukuba, Japan*

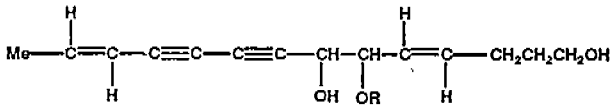
Hairy roots of Korean balloon flower (*Platycodon grandiflorum* A. DC) were induced from the root tissues infected with *Agrobacterium rhizogenes* ATCC 15834. Growth and polyacetylene [lobetyol (1), lobetyolin (2) and lobetyolinin (3)] production of ten hairy root clones cultured in 1/4 Gamborg B5 (B5) 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 1 (0.375% dry wt) and at week 7 for 2 and 3 (3.030% and 0.206% dry wt, respectively) whose levels were much higher than those of the intact plant root (1: 0.019%, 2: 0.077% dry wt, 3 was not detected).

Keywords : *Platycodon grandiflorum*, Campanulaceae, hairy root, *Agrobacterium rhizogenes*, polyacetylene, lobetyol, lobetyolin, lobetyolinin

Platycodon grandiflorum A. DC (Campanulaceae) 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 (Tada *et al.*, 1975; Ishii *et al.*, 1978, 1984; Konishi *et al.*, 1978), 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 inflanta* by Ishimaru *et al.* (1991, 1994) and named as lobetyol (**1**), lobetyolin (**2**) and lobetyolinin (**3**). It was reported that the content of these polyacetylenes in hairy root culture of *L. inflanta* was higher than that in the roots of the intact plants (Ishimaru *et al.*, 1992, 1993). In *L. sessilifolia* (Ishimaru *et al.*, 1994) and *P. grandiflorum* (Tada *et al.*, 1995) this fact was also demonstrated. In addition it has been reported that these polyacetylenes can be used as important chemotaxonomic markers in campanulaceous plants (Tada *et al.*, 1995).

We describe here 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.



1. lobetyol : R=H
2. lobetyolin : R=Glc
3. lobetyolinin : R=Glc 6 1 -Glc

MATERIALS AND METHODS

Establishment of *P. grandiflorum* hairy root cultures

Platycodon grandiflorum roots were purchased at

*Corresponding author: Fax +82-2-820-5206

a market in Kwangju. They were sterilized with a NaOCl solution (available chlorite concentration of 3%) containing Tween 20 for 15 min and followed by washing three times with distilled water. Roots were then aseptically cut into approximately 1 cm thickness. There disinfected root disc were used for *Agrobacterium infection*. *A. rhizogenes* ATCC 15834 subcultured on YEB (Vervliet *et al.*, 1975) 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 Gamborg B5 (B5) medium (Gamborg *et al.*, 1968) supplemented with 0.75% agar and 0.5 mg/mL Claforan, an antibiotic for the elimination of bacteria. The axenic hairy roots were transferred to hormone-free 1/4 B5 liquid medium or 1/10 Murashige-Skoog [(MS), Murashige and Skoog, 1962] (one tenth macro elements of MS basal medium, 30 mL per 100 mL flask) liquid medium 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. The 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 method with *rol* A-1, *rol* B-2 (for TL) and AGS-1, AGS-2 (for TR) as primers.

Selection of high polyacetylene-yielding clone

Ten hairy root clones of *P. grandiflorum* (ca. 100 mg, fresh wt) were inoculated into 1/4 B5 liquid medium and cultured at 25℃ in the dark (100 rpm, on a rotary shaker). The hairy roots were harvested after 4 weeks of culture and the growth (fresh and dry wt) and polyacetylene content were detected.

Medium for optimal growth and high polyacetylene production of clone D6

Hairy root clone D6 (ca. 100 mg, fresh wt) was inoculated into three different hormone free-liquid media, MS, B5, Woody Plant (WP) (Lloyd and McCown, 1980) 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). The hairy roots were harvested after 4 weeks of culture and the growth (fresh and dry wt) and polyacetylene content were 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 250C in the dark (100 rpm, on a rotary shaker). The hairy roots were harvested weekly (1-7 weeks) and fresh wt, dry wt and polyacetylene content were determined.

Analysis of polyacetylenes

Sample preparation and HPLC conditions for the quantification of polyacetylenes (**1**-**3**) were the same as described by Ishimaru *et al.*, (1993).

RESULTS AND DISCUSSION

For hairy root induction of *P. grandiflorum*, the edible roots sold at a market were used for the infection of *Agrobacterium rhizogenes* ATCC 15834. Tips of hairy roots induced on the root discs were individually excised and cultured on 1/4 B5 or 1/10 MS solid medium. Although some differences in growth pattern, such as growth rate, density of root hairs and branching ability on solid media, were observed, ten clones (D1-10) showing fast growth in several passages were selected and used in this study. In

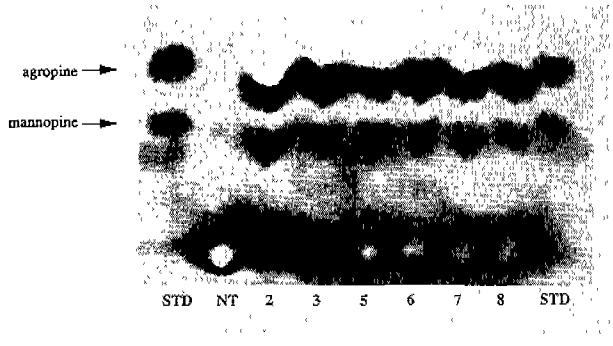


Fig. 1. Opine assay of *P. grandiflorum* hairy root clones transformed with *A. rhizogenes* ATCC 15834. STD, agropine and mannopine; NT, non-transformed roots. Numbers represent the hairy root clones.

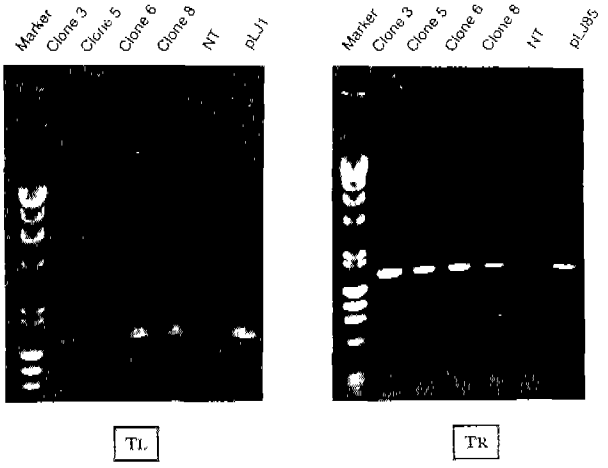


Fig. 2. Polymerase chain reaction analysis of *P. grandiflorum* hairy root clones transformed with *A. rhizogenes* ATCC 15834. Marker, λ*Han*dIII+φX*Hae*III digestion; NT, non-transformed roots. pLJ1 and pLJ85 (cosmid) are positive control of TL- and TR-DNA, respectively.

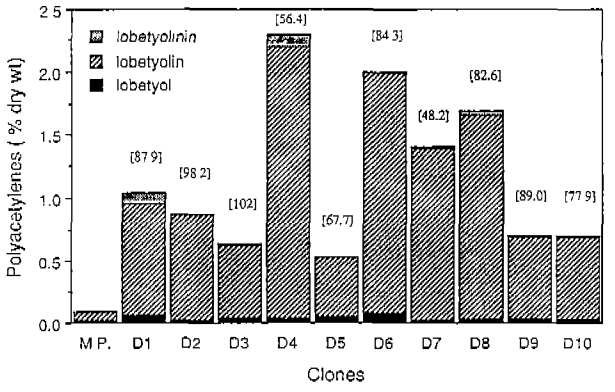


Fig. 3. Growth and polyacetylene production of *P. grandiflorum* hairy root clones cultured in 1/4 B5 liquid medium for 4 weeks at 25° in the dark. Values in brackets show dry wt (mg per 100 mL flask). M.P. represents the root of mother plant used for hairy root induction.

all selected clones the integration of both TL- and TR-DNAs was confirmed by the detection of opines (Fig. 1) and polymerase chain reaction (PCR) analysis (Fig. 2).

To select the high polyacetylene-yielding clone, growth and polyacetylene content of ten clones after culturing in 1/4 B5 liquid medium for 4 weeks were determined (Fig. 3). 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. Amongst these clones, D6 which showed

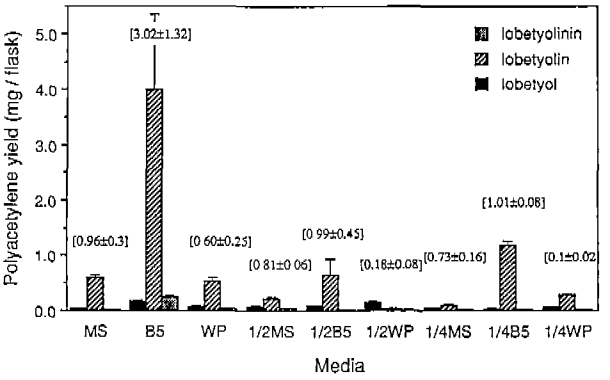


Fig. 4. Growth and polyacetylene production of *P. grandiflorum* hairy root clones D6 cultured in various liquid medium for 4 weeks at 25° in the dark. Values in brackets show the fresh weight (mg per 100 mL flask± standard error). Bars represent standard errors.

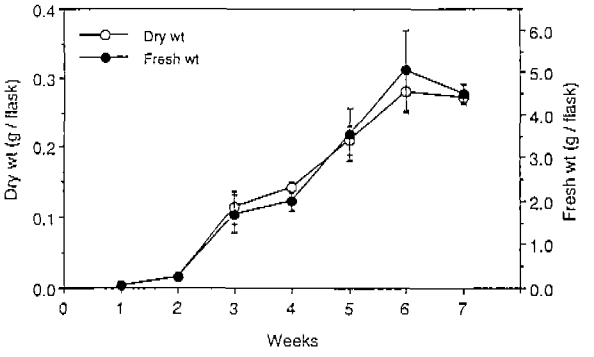


Fig. 5. Growth of *P. grandiflorum* hairy root clones D6 cultured in B5 liquid medium for 7 weeks at 25° in the dark. Bars represent standard errors.

good growth (84.3 mg per flask) and polyacetylene production (2.0% dry wt of **1**-**3**) was selected for following experiments.

For the determination of the optimal medium for the growth and polyacetylene production of D6, various liquid media (MS, B5, WP), half strength of MS, B5, WP and one fourth strength of MS, B5, WP) were tested. As shown in Fig. 4, B5 medium was best for growth (3.02 g, fresh wt per flask) and polyacetylene production (**1**: 0.24 mg, **2**: 3.99 mg, **3**: 0.16 mg per flask).

The time course (1-7 weeks) of the growth and polyacetylene production of the clone D6 in B5 liquid medium was also investigated. The rapid growth, started after week 2, continued until week 6 when the highest root weight (fresh wt: 5.06 g, dry wt: 0.28 g per flask) was observed (Fig. 5). The pro-

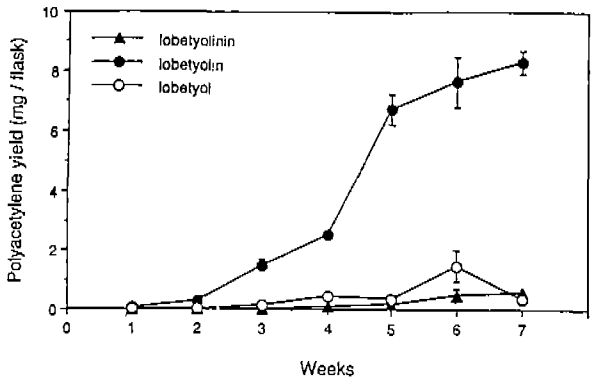


Fig. 6. Polyacetylene production in *P. grandiflorum* hairy root clones D6 cultured in B5 liquid medium for 7 weeks at 25° in the dark. Bars represent standard errors.

duction of polyacetylenes (in particular 2) roughly paralleled the root growth showing the 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. 6). The content of these polyacetylenes (% dry wt) in clone D6 was about 40 times higher than that in parent plants (**1**: 0.02%, **2**: 0.08% and **3** was not detected) which had used for hairy root induction.

The maximum polyacetylene yield of hairy root (D6) induced from the Korean balloon flower was almost 5 times larger than that of the hairy roots (induced by *A. rhizogenes* MAFF 03-01724) from Japanese one (Tada *et al.*, 1995). This result demonstrated the importance of the selection of hairy root clones (together with *Agrobacterium* strains used for hairy root induction) and the 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 analysis may be required from the view of taxonomy.

ACKNOWLEDGEMENTS

The authors thank Dr. L. Jouanin for providing pLJ 1 and pLJ 85 and Mr. Hideki Matsumoto for PCR analysis and Ms. W. S. Shu for critical reading of our manuscript. This work was supported in part by the Hormone Research Center (HRC-95-0202-3).

LITERATURE CITED

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell. Res.* **50**: 151-158.

Ishii, H., K. Tori, T. Tozyo and Y. Yoshimura. 1978. Structures of polygalacin-D and -D2, and their monoacetates, saponins isolated from *Platycodon grandiflorum* A. DC., determined by carbon-13 nuclear magnetic resonance spectroscopy. *Chem. Pharm. Bull.* **26**: 674-677.

Ishii, H., K. Tori, T. Tozyo and Y. Yoshimura. 1984. Saponins from roots of *Platycodon grandiflorum*. Part 2. Isolation and structure of new triterpene glycosides. *J. Chem. Soc Perkin Trans. I*, 661-668.

Ishimaru, K., H. Arakawa, M. Yamanaka and K. Shimomura. 1994. Polyacetylenes in *Lobelia sessilifolia* hairy roots. *Phytochemistry.* **35**: 365-369.

Ishimaru, K., H. Arakawa, S. Sadoshima and Y. Yamaguchi. 1993. Effects of basal media on growth and polyacetylene production of *Lobelia inflanta* hairy roots. *Plant Tissue Cul. Lett.* **10**: 191-193.

Ishimaru, K., H. Yonemitsu, and K. Shimomura. 1991. Lobetyolin and lobetyol from hairy root culture of *Lobelia inflanta*. *Phytochemistry.* **30**: 2255-2257.

Ishimaru, K., S. Sadoshima, S. Neera, K. Koyama, K. Takahashi and K. Shimomura. 1992. A polyacetylene gentiobioside from hairy roots of *Lobelia inflanta*. *Phytochemistry* **31**: 1577-1579.

Konishi, T., A. Tada, J. Shiji and O. Tanaka. 1978. The structures of platycodin A and C, monoacetylated saponins of the roots of *Platycodon grandiflorum* A. DC. *Chem. Pharm. Bull.* **26**: 668-670.

Lloyd, G. B. and B. H. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Int. Plant. Prop. Soc. 30: 421-427.*

Murashige, T and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* **15**: 473-497.

Tada, A., Y. Kaneiwa, J. Shoji and S. Shibata. 1975. Studies on the saponins of the root of *Platycodon grandiflorum* A. De Candolle. I. Isolation and the structure of platycodin-D. *Chem. Pharm. Bull.* **23**: 2965-2972.

Tada, H., K. Shimomura and K. Ishimaru. 1995. Polyacetylenes in *Platycodon grandiflorum* hairy root and Campanulaceous plants. *J. Plant Physiol.* **145**: 7-10.

Vervliet, G., M. Holsters, H. Teuchy, M. van Montague and J. Schell. 1975. Characterization of different plaque-forming and defective temperate phages in *Agrobacterium strains. J. Gen. Virol.* **26**: 33-48.

(Received October 6, 1995)

도라지(*Platycodon grandiflorum*) 뿌리組織에서 高濃度 Polyacetylene 含有 毛狀根의 誘導 및 培養

安 埈 徹 · 蘇 祥 燮[1] · Koichiro Shimomura[2] · 黃 柏*
全南大學校 自然科學大學 生物學科 및 호르몬연구센타
[1]全北大學校 生物敎育學科, [2]日本 쓰꾸바 藥用作物栽培試驗場

적 요

도라지(*Platycodon grandiflorum* A. DC)의 모상근이 *Agrobacterium rhizogenes* ATCC 15834의 감염에 의해 뿌리 조직으로부터 유도되었다. 유도·선발된 10 모상근 클론의 생장과 polyacetylene [lobetyol (**1**), lobetyolin (**2**) and lobetyolinin (3)] 생산이 1/4 Gamborg B5 (B5) 액체배지를 사용하여 검토되었다. 선발된 클론 D6는 호르몬 무첨가 B5 액체배지에서 생장이 양호하였으며, polyacetylenes의 최대함량은 배양 6주째에 **1**이 (0.375% 건중량)와 배양 7주째에 **2**와 **3** (**2**: 3.030%와 **3**: 0.206% 건중량)이 각각 최대치를 보여 모상근 유도에 사용한 모식물(**1**: 0.019%, **2**: 0.077% 건중량, **3**은 비검출)에 비하여 훨씬 높은 함량을 보여주었다.

주요어 : 도라지, 모상근, *Agrobacterium rhizogenes*, polyacetylene, lobetyol, lobetyolin, lobetyolinin

*교신저자 : Fax (02) 820-5206