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POLYACETYLENE GLUCOSIDES IN HAIRY ROOT CULTURES OF *LOBELIA CARDINALIS*

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Key Word Index—*Lobelia cardinalis*; Campanulaceae; hairy root culture; *Agrobacterium rhizogenes*; polyacetylene; glucoside; lobetyol; lobetyolin; lobetyolinin.

Abstract—Hairy root cultures of *Lobelia cardinalis* clones Lc-A, induced by *Agrobacterium rhizogenes* ATCC 15834, and Lc-M, induced by *A. rhizogenes* MAFF 03-01724, were established. In hormone-free Murashige–Skoog liquid medium (50 ml medium per flask), both clones yielded high amounts of the polyacetylene glucosides, lobetyolin (18.7 mg per flask, Lc-M) and lobetyolinin (24.7 mg per flask, Lc-A), whose production level was the maximum in all cultures investigated.

INTRODUCTION

Lobelia cardinalis, native to middle eastern parts of North America (south region of Canada and Texas), is generally used as a garden plant (usually on damp ground) and as an aquatic plant which decorates ornamental tanks for tropical fish. In spite of its popularity for horticultural use, there have been few studies on the secondary metabolites of this species. Recently, Tada *et al.* reported the existence of polyacetylene constituents in intact plants [1]. In the course of our chemical studies on campanulaceous plants, hairy root cultures (transformed with *Agrobacterium rhizogenes*) of *L. cardinalis* were established and found to yield high amounts of polyacetylene glucosides.

RESULTS AND DISCUSSION

In vitro grown plants of *L. cardinalis* were infected with two types of *A. rhizogenes* strains (MAFF 03-01724 and ATCC 15834). Among several hairy roots induced from the infected tissues, nine clones (seven from MAFF 03-01724 and two from ATCC 15834) were selected and cultured in hormone-free 1/2 Murashige–Skoog (MS) liquid medium [2]. Two clones of Lc-A (induced by *A. rhizogenes* ATCC 15834) and Lc-M (induced by *A. rhizogenes* MAFF 03-01724), which showed good growth in the medium, were used for our experiments.

Firstly, the growth of Lc-A and Lc-M in various hormone-free {MS, 1/2 MS, Gamborg B5 (B5) [3] and Woody Plant (WP) [4]} liquid media was determined (Fig. 1). Lc-A started to proliferate at the early stage of the culture period in all media. The fresh weight of roots rapidly increased to reach a maximum at week 8: in MS, 8.24 g; in 1/2 MS, 9.12 g; in B5; 7.87 g; and in WP, 9.18 g per flask. Lc-M cultured in WP medium grew rapidly until the third week when compared with other media. Both in MS and 1/2 MS media, the growth of Lc-M was similar to that of Lc-A observed in the four media. In contrast, when Lc-M was cultured in B5 medium, the weight gradually increased and, after week 5, it increased much more rapidly. The other clones induced by *A. rhizogenes* MAFF 03-01724 also showed similar growth patterns in these four media (data not shown). Therefore, the difference of the growth patterns between Lc-A and Lc-M observed in B5 and WP media appear to have originated from the dissimilarity of the bacteria (Ri-plasmids) infected.

The hairy root cultures of some campanulaceous plants [1, 5, 6] are well known to yield the polyacetylene constituents, lobetyol [7], lobetyolin [7] and lobetyolinin [8]. Production of these polyacetylenes in Lc-A (Fig. 2) and Lc-M (Fig. 3, only in MS medium) cultured in four basal liquid media was determined. In both cultures, the following results were obtained. (a) The maximum amount of polyacetylenes was observed after six to eight weeks of cultures (e.g. 24.7 mg per flask of lobetyolinin in Lc-A at week 6 and 18.7 mg per flask of lobetyolin in Lc-M at week 8, both in MS medium); (b) the amount of lobetyol was constantly low (below 2 mg per flask); (c) in 1/2 MS, B5 and WP media, the amounts of polyacetylenes produced by Lc-A and Lc-M were almost identical, although Lc-M in B5 medium showed a short lag-period (one to four weeks). The high production of lobetyolin and lobetyolinin in these

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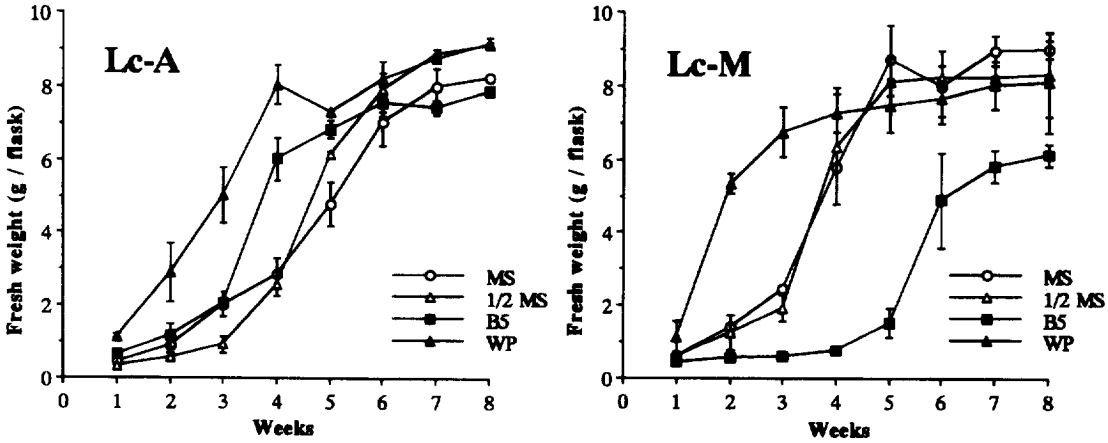


Fig. 1. Growth of *Lobelia cardinalis* hairy roots (Lc-A and Lc-M) cultured in four basal liquid media. (Bars represent standard errors).

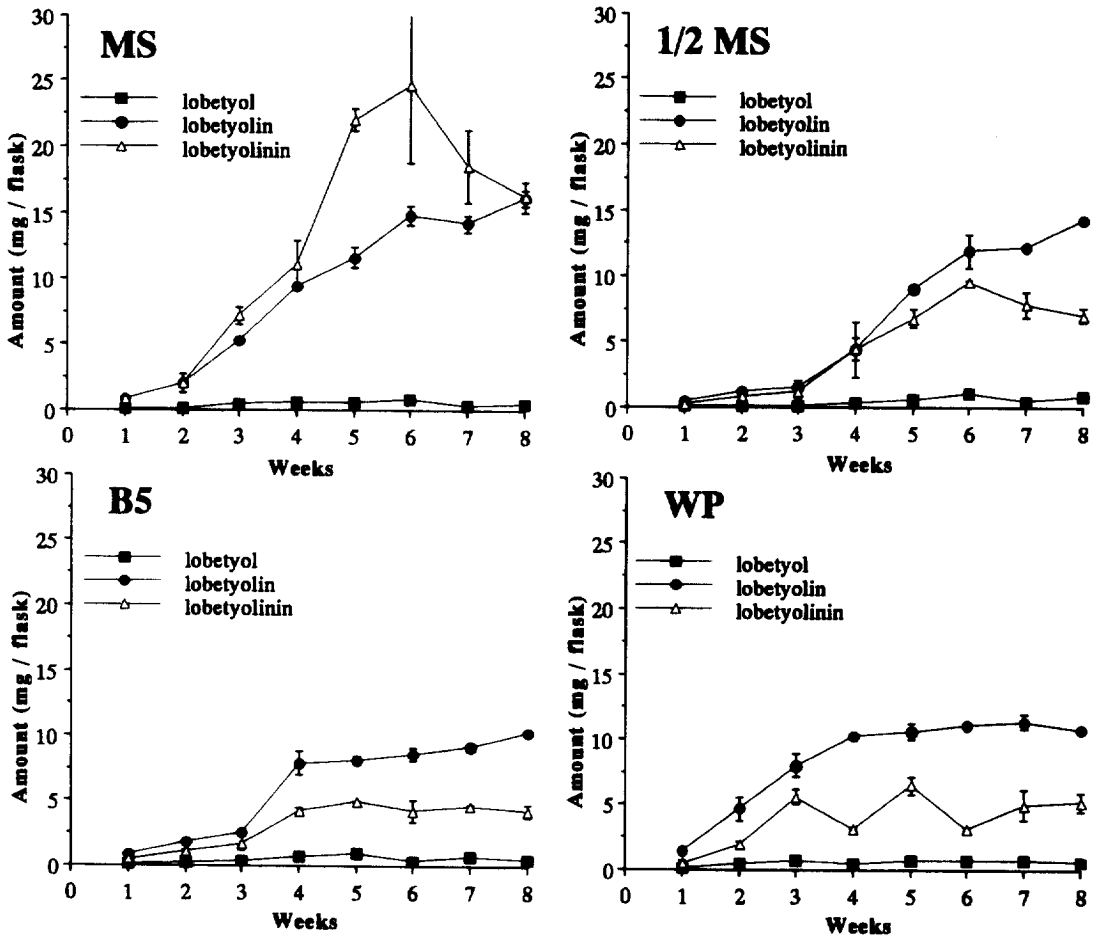


Fig. 2. Polyacetylene production in *Lobelia cardinalis* hairy roots (Lc-A) cultured in four basal liquid media. (Bars represent standard errors).

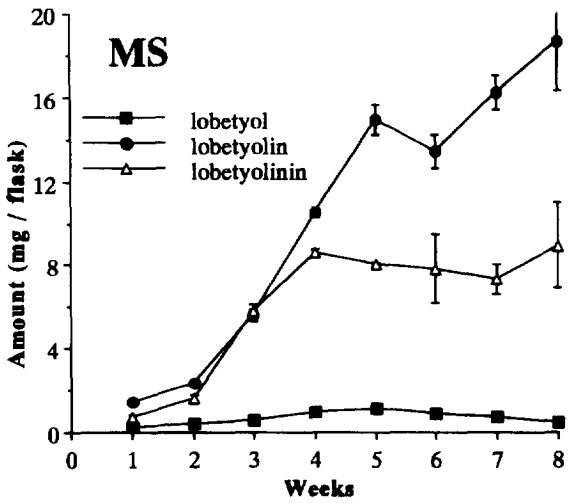
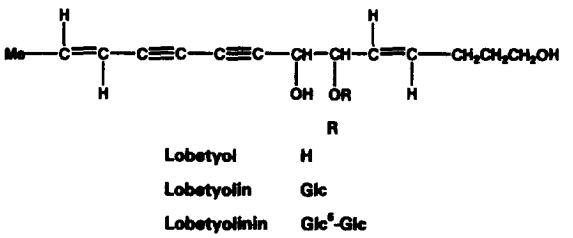


Fig. 3. Polyacetylene production in *Lobelia cardinalis* hairy roots (Lc-M) cultured in MS liquid media. (Bars represent standard errors).



cultures showed the strong capability for glucosylation in these hairy roots.

Among the various tissue cultures of campanulaceous plants we have investigated so far, *L. cardinalis* hairy roots appear to be best for the biosynthetic study of glucosyl metabolism, as well as for the production of polyacetylene glucosides.

EXPERIMENTAL

All culture media, MS, 1/2 MS (containing 30 $g l^{-1}$ sucrose), B5 (containing 20 $g l^{-1}$ sucrose) and WP (containing 20 $g l^{-1}$ sucrose) were adjusted to pH 5.7 before autoclaving at 121° for 15 min. All cultures were grown at 25°. Data shown are the mean of three experiments.

Plant material. L. cardinalis L. plants were purchased from a market. Nodal segments were sterilized (2% NaOCl) and placed aseptically on hormone-free MS solid medium (solidified with 2.5 $g l^{-1}$ gelrite) to establish shoot culture. Axenic plants *in vitro* were used for *Agrobacterium* infection.

Hairy root cultures. A. rhizogenes ATCC 15834 and *A. rhizogenes* MAFF 03-01724, subcultured on YEB agar medium [9], were inoculated using a needle on to the cut ends of the stems (*ca* 1 cm length) prepd from axenic shoot cultures. About 20 days after infection, several hairy roots appeared at the inoculated sites (in the dark condition). Hairy roots were cut off and placed on hormone-free 1/2 MS solid medium containing claforan, 0.5 $mg ml^{-1}$, to eliminate the bacteria. Nine clones (7 from MAFF 03-01724 and 2 from ATCC 15834) of the axenic hairy roots were transferred to hormone-free 1/2 MS liquid medium (50 ml per 100-ml flask) and cultured on a rotary shaker (100 rpm) in the dark. Two clones (Lc-A and Lc-M) were selected and used for expts. Transformation of hairy roots was established by detection of opines (agropine and mannopine [10] from Lc-A and mikimopine [11] from Lc-M) using paper electrophoresis. Voucher specimens are deposited at the Faculty of Agriculture, Saga University.

Hairy root cultures in different media. Lc-A and Lc-M (*ca* 0.5 g, fr. wt) were inoculated into 4 hormone-free liquid media (MS, 1/2 MS, B5 and WP, 50 ml per 100-ml flask) and cultured (100 rpm, on a rotary shaker) in the dark. Growth (root wt) and polyacetylene production were determined once a week for 8 weeks.

HPLC analysis for polyacetylenes. Sample prepn and HPLC conditions for determination of polyacetylenes were the same as described in ref. [5].

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REFERENCES

1. Tada, H., Shimomura, K. and Ishimaru, K. (1995) *J. Plant Physiol.* **145**, 7.
2. Murashige, T. and Skoog, F. (1962) *Physiol. Plant.* **15**, 473.
3. Gamborg, O. L., Miller, R. A. and Ojima, K. (1968) *Exp. Cell Res.* **50**, 151.
4. Lloyd, G. B. and McCown, B. H. (1980) *Int. Plant. Prop. Soc.* **30**, 421.
5. Ishimaru, K., Arakawa, H., Sadoshima, S. and Yamaguchi, Y. (1993) *Plant Tissue Cult. Letters* **10**, 191.
6. Ishimaru, K., Arakawa, H., Yamanaka, M. and Shimomura, K. (1994) *Phytochemistry* **35**, 365.
7. Ishimaru, K., Yonemitsu, H. and Shimomura, K. (1991) *Phytochemistry* **30**, 2255.
8. Ishimaru, K., Sadoshima, S., Neera, S., Koyama, K., Takahashi, K. and Shimomura, K. (1992) *Phytochemistry* **31**, 1577.
9. Vervliet, G., Holsters, M., Teuchy, H., van Montague, M. and Schell, J. (1975) *J. Gen. Virol.* **26**, 33.
10. Petit, A., David, C., Dahl, G. A., Ellis, J. G., Guyon, P., Casse-Delbart, F. and Tempé, J. (1983) *Mol. Gen. Genet.* **190**, 204.
11. Isogai, A., Fukuchi, N., Hayashi, M., Kamada, H., Harada, H. and Suzuki, A. (1990) *Phytochemistry* **29**, 3131.