Notes Plant Tissue Culture Letters, **13**(1), 85-86(1996)

Anthocyanins in *Lobelia chinensis* Hairy Roots

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Lobelia chinensis Lour., a campanulaceous weed widely grown in East Asian countries, has been a medicinal plant (used in China) for removal of fever (via detoxication and diuresis) and treatments of schistosomiasis and liver cirrhosis. Although the alkaloid (lobeline) production in the plant has been well-known, the detailed study on the secondary metabolites has not been done. Recently, the production of three polyacetylenes, lobetyol, lobetyolin and lobetyolin in the intact plant[1] and in the hairy root cultures[2] induced by the infection with *Agrobacterium rhizogenes* ATCC 15834 was reported. The hairy roots, cultured under light condition, also accumulated chlorophylls (a and b) in four basal liquid media[2], *i. e*, Murashige-Skoog[3] (MS), Gamborg B5[4] (B5), Woody Plant[5] (WP) and Root Culture[6] (RC). In addition, the cultures in these media (only under light condition) were found to yield some reddish purple pigments. In the present study, the pigments produced in the hairy roots were identified and the contents in media (MS, B5, WP and RC) were determined by HPLC.

The hairy roots, cultured in four basal liquid media for 8 weeks in the light (16 hr photoperiod/day, 60 μmol/m^{2}s), were lyophilized and extracted [*ca.* 50 mg, dry weight (dw)] with 1% HCl-MeOH (1 m*l*) for 1 day at room temperature in the dark. The extract, after filtration through filter paper (ADVANTEC, 5 B), was applied (15 μl) to HPLC analysis. HPLC conditions were as follows: column; Inertsil ODS-2 (4.6 mm i. d. × 250 mm), mobile phase; A: 1.5% H_3PO_4, B: 1.5% H_3PO_4-20% CH_3COOH-25% CH_3CN (A : B = 3 : 1→13 : 7, in 30 min.), flow rate; 1.0 m*l*/min., column temperature; 35°C, detection; 520 nm. Two compounds, Lc-1 [retention time (R_t) 10.5 min.] and Lc-2 (R_t 12.0 min.) observed in the HPLC chromatogram (**Fig. 1**), were identified as cyanidin 3-*O*-glucoside[7] and cyanidin 3-*O*-rutinoside[8], respectively, by direct comparison of the R_ts with those

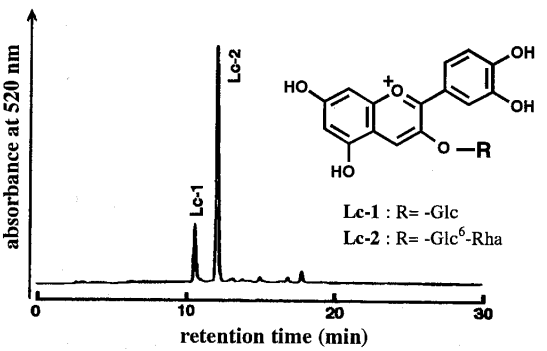

Fig. 1 HPLC profile of 1% HCl-MeOH extract of *Lobelia chinensis* hairy roots cultured in RC medium for 8 weeks in the light.

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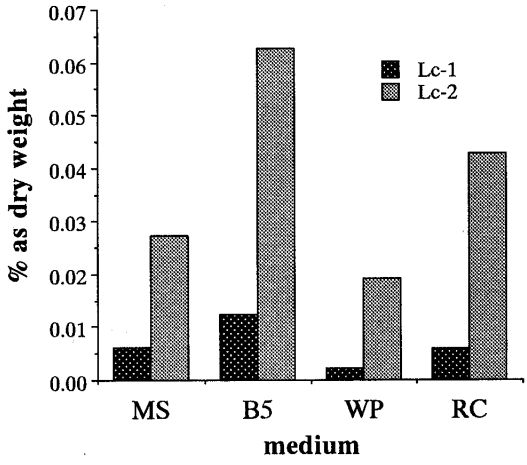

Fig. 2 Anthocyanin contents in *Lobelia chinensis* hairy roots cultured in four basal liquid media.
Data are shown as the mean of duplicate experiments.

of the authentic samples.

The reddish purple pigments produced in the hairy roots were, for the most part, Lc-1 and Lc-2 in all media tested. Particularly, the content of Lc-2 was higher (*ca.* 4-8 times) than that of Lc-1 (**Fig. 2**). The maximum contents of Lc-1 (0.012%) and Lc-2 (0.063%) were observed in B5 medium. In the hairy root cultures of *L. chinensis*, Tada *et al.*[2] reported the high production of chlorophylls in the media with high ratio of NO_3^-/NH_4^+ such as B5 and RC. Our results showed that the anthocyanin production in the hairy roots also has a tendency to be promoted in these media (B5 and RC) similar to the case of chlorophyll metabolism. This is the first report on the production of anthocyanins in various tissue cultures of campanulaceous plants and is also very helpful for future determination of the pigments in petal portion of *L. chinensis* intact plant whose chemical structures remaining obscure.

Acknowledgements

This work was supported in part by the subsidy from The San-Ei Gen Foundation for Food Chemical Research and by the Ministry of Health and Welfare, Science Research Fund Subsidy granted to the Japan Health Science Foundation. (Accepted October 28, 1995)

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