

Rosmarinic Acid and Related Phenolics in Adventitious Root Cultures of *Ocimum basilicum* L.

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Rosmarinic acid (RA), a natural phenolic antioxidant, is one of the most common caffeic acid esters occurring particularly in Labiatae and Boraginaceae. Numerous investigations on RA production in these families, in plants as well as in the cultured cells, have been done mainly in the genera of *Coleus*¹⁾, *Salvia*²⁾, *Rosmarinus*³⁾ (Lamiaceae), *Lithospermum*⁴⁾, *Anchusa*⁵⁾ (Boraginaceae) etc. *Ocimum basilicum* L., a lamiaceous plant being well-known as "Sweet Basil", is a very popular kitchen herb which is also suitable as an ornamental garden plant. Although the RA production in *O. basilicum* L. (together with several herbs) has been analyzed⁶⁾, further studies on the secondary metabolism of this plant accompanied by tissue culture techniques have not been done. In the present experiment, the adventitious root cultures of this plant were firstly established and the chemical constituents [RA and related phenolics lithospermic acid⁷⁾ (LA) and lithospermic acid B⁸⁾ (LAB)] were investigated.

The seeds of *O. basilicum* were sterilized by the usual method (with 2% NaOCl, 8 min.) and germinated aseptically on Murashige-Skoog (MS) solid medium⁹⁾ (2.5 g/l gelrite). The roots of the axenic plants *in vitro* were cut, transferred into MS liquid medium (50 ml in 100 ml flask) containing 1 mg/l NAA and maintained in the dark at 25°C on a rotary shaker. The root cultures thus established and subcultured for a year were used for this experiment. After inoculation (ca. 300 mg, fresh weight) into the medium, the biomass of the roots linearly increased at the early stage of the culture period (until week 4) (Fig. 1a). At the later period of the culture, the roots gradually proliferated to reach the maximum amount (668.5 mg, as dry weight/a flask) at week 7.

RA and related phenolics' contents in the roots were analyzed by HPLC. The lyophilized roots (ca. 20 mg) were mashed and extracted with MeOH (2 ml) for 16 hr at room temperature. Each extract, after filtration through a millipore filter (0.5 µm), was subjected to HPLC analysis; column: CAPCELL PAK C18 AG 120 (4.6 mm × 250 mm), mobile phase; 1 mM tetra-butylammonium (adjusted to pH 2.8 with AcOH) - CH₃CN (4 : 1 → 1 : 4, in 25 min.), flow rate: 0.65 ml/min., column temperature: 40°C, detection: 330 nm, R_t (min.) : RA (15.5), LA (20.7) and LAB (22.5). *O. basilicum* root cultures produced both RA and LA, particularly showing continuous increment of RA

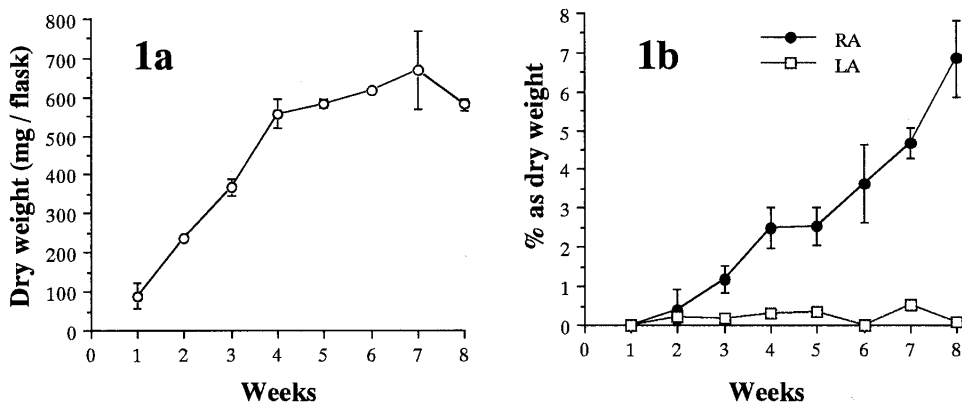


Fig. 1 Growth [1a, ca. 300 mg (fresh weight) of roots were inoculated] and rosmarinic acid (RA) and lithospermic acid (LA) contents (1b) of the adventitious root cultures of *Ocimum basilicum* L. cultured in MS liquid medium (50 ml in 100 ml flask) containing 1 mg/l NAA.

Data are shown as the mean of duplicate experiments, bars represent standard errors.

content throughout the culture time (Fig. 1b, RA was the major phenolic produced in the cultures), although LAB was not detected in the cultures. The maximum contents of RA and LA, observed at the later period of the culture (6.84% RA at week 8 and 0.52% LA at week 7, as dry weight), were relatively higher than those of the intact plant (RA: 3.98%, LA: not detected, leaf portion) and *in vitro* shoots (RA: 5.01%, LA: 0.36%, leaf portion) cultured on MS solid medium in the light (16 hr photoperiod/day, ca. 5000 lux). The calculative maximum weights of RA and LA obtained in a flask (100 ml volume containing 50 ml medium) in the root cultures reached to 55.3 mg (RA at week 8) and 3.4 mg (LA at week 7). Although LAB, a tetrameric derivative of caffeic acid, was not detected in the root cultures, these adventitious roots seem to be suitable for the biosynthetic study of caffeic acid metabolism (by some elicitor experiments *etc.*) as well as for the production of RA.

At this time, most research on RA production in tissue cultures of Lamiaceae and Boraginaceae have been done using their cell suspension cultures^{1,2,4,5}. This is the first report on the establishment of root cultures of *O. basilicum* accompanied by high production of these antioxidant polyphenols (RA and LA), the most common caffeic acid derivatives.

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《和文要約》

スイートバジル不定根培養におけるローズマリー酸と関連フェノール類の生産

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1 mg/l NAA 添加 Murashige-Skoog 液体培地で培養したスイートバジル (*Ocimum basilicum* L.) 不定根は、高含量 (6.84%, 8 週目, 乾燥重量あたり) で rosmarinic acid を生産した。また、その関連フェノールである lithospermic acid も少量 (0.52%, 7 週目, 乾燥重量あたり) 認められたが、lithospermic acid B の生産は確認されなかった。不定根培養における rosmarinic acid や lithospermic acid の含量は、親植物体や茎葉培養体の葉部における含量よりも高い値であった。