



Production of Tanshinones and Phenolic Acids in Hairy Root Cultures of *Perovskia abrotanoides* Karel.

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Perovskia abrotanoides Karel. (Lamiaceae), with vernacular name of Brazambal in Iran, contains two major classes of secondary metabolites (diterpenoid tanshinones and phenolic acids), which are responsible for the pharmacological activities of the plant. Ability to synthesize a wide range and a large amount of secondary metabolites are the most important advantages of hairy root cultures. The main purpose of this study was to investigate the production of biomass, tanshinones and phenolic acids in hairy root lines of *P. abrotanoides*. Mature seeds were collected from wild plants and were cultured on free hormone MS medium. *Agrobacterium rhizogenes* strain A4 was used to infect the nodes on one-month old plants of *P. abrotanoides*. Then infected plants were cultured on MS medium for 48h at 25°C under 16:8 light: dark period. Transgenic nature of regenerated roots was confirmed by PCR amplification of *rolC* as the target gene. The hairy root line A4L2 was selected for further experiments. The hairy roots were harvested at intervals of 4 days and their fresh and dry weights were measured. The contents of phenolic acids and tanshinones in the hairy roots were determined by HPLC method. The growth curve and metabolites accumulation patterns of hairy roots were obtained during a 40-day growth cycle. Quantitative analyses indicated that the whole growth cycle could be divided into four stages: adaptive phase (0-4th day), exponential phase (4-24th day), stationary phase (24-36th day), and decline phase (after the 36th day). The highest biomass (3.90g FW and 0.37g DW) accumulation, as well as the maximum contents of rosmarinic acid (5.88 mg/g DW), salvianolic acid A (0.47 mg/g DW), salvianolic acid B (12.72 mg/g DW), cryptotanshinone (2.61 µg/g DW), tanshinone I (1.54 µg/g DW) and tanshinone IIA (2.86 µg/g DW) were achieved on the 36th day. The amounts of rosmarinic acid, salvianolic acids A and B in hairy roots were increased by 49.40, 78.77 and 43.69%, respectively, as compared to the intact roots, however no significant increases were found in tanshinones contents. Our findings indicated that hairy root culture could be a promising tool to produce tanshinones and phenolic acids in *P. abrotanoides*.

Keywords: *Perovskia abrotanoides* Karel., *Agrobacterium rhizogenese*, Hairy root culture,