Abstract

Catharanthus roseus is one the main pharmaceutical plant from Apocynaceae family, which is able to produce pivotal indole alkaloids such as vincristin and vinblastin. Increasing demand on this compound as an anti leukemia resulted in a great interest. Hairy root cultures which are produced by *Agrobacterium rhizogenes*, produce a huge amount of the extended root as a main source of secondary metabolites in a short time. In this study, the effective factors in production, preservation, stability and growth of hairy roots were surveyed for increasing vincristin. Four strains: 2659, 10266, 15834, A4 and three explants (*in vitro* leaf and stem samples and *ex vitro* leaf) were applied for transformation. The best strain and explant were selected for rest of study such as the effect of bacteria culture medium and coculture medium. Transgenic hairy roots were confirmed by specific primers of *rol*C and *vir* genes. Transgenic hairy roots were cultured in three cultures conditions: solid culture, liquid culture with shaking, and liquid culture without shaking. HPLC analysis was used for determination of vincristin. Our study showed that maximum rate of callus and hairy root productions were accured by 10266 strain and *in vitro* leaf. ½B5 medium was significantly better for hairy root production. Also the YMB+MS as bacterial medium was more effective in hairy root appearance. Further the best culture condition for hairy root establishment was liquid without shaking. HPLC analysis showed that vincristin in hairy roots was 2.5 times of that in normal roots and 1.5 times of that in shoots. Our study reveals that bacterial strain, explants and medium culture can be effective on production of hairy roots and with optimization of condition can improve the production more amount of vincristin.

Key words: Bacterial strain, coculture medium, hairy root, HPLC, vincristin.