GENETIC STUDIES ON PRODUCTION OF SOME SECONDARY METABOLITES IN MEDICINAL PLANTS

By

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B.Sc. Agric. Sc. (Genetics), Ain Shams University, 2005

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صفحة الموافقة على الرسالة

دراسات وراثية على إنتاج بعض المركبات الثانوية فى النباتات الطبية

رسالة مقدمة من

منـــي محـــمد مغـــازي علـــي دراز

بكالوريوس علوم زراعية (وراثة)، جامعة عين شمس، ٢٠٠٥

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ABSTRACT

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The Madagascar periwinkle *Catharanthus roseus* (L.) G. Don (Apocynaceae) produces a wide range of monoterpenoid indole alkaloids (MIAs). Some of these secondary metabolites possess therapeutical value. Monomeric MIAs, ajmalicine and serpentine are used in the treatment of hypertension, while the dimeric MIAs, vincristine and vinblastine, are powerful anti-cancer drugs in widespread use in cancer chemotherapy. Tissue culture of *C. roseus* has been considered to be sources of medicinally important MIAs, but have suffered from low productivity.

A protocol for the establishment of *in vitro* tissue cultures of *C. roseus* is described. Callus was initiated from mature leaf explants on MS medium supplemented with source at a concentration of 30 g/L and 1 mg/L of 2, 4 D + 0.1 mg/L of Kinetin, which proved to be more appropriate for callus induction and growth of the Egyptian *C. roseus* and routinely used in this study for callus production and as a control medium in the different treatment experiments. Cultures were incubated in 16 light and 8 dark at 22-25°C. All culture media used in this study were adjusted to pH= 5.6 - 5.8 before solidification with 0.2% gel rite.

In this study, three different sucrose concentrations (40, 50, and 60 g/L) and three concentrations of benzyl adenine (0.1, 0.2 and 0.4mg/L) in addition to two concentrations of jasmonic acid (10 μM and 100 μM) were studied to determine their influence on growth and alkaloid formation in *C. roseus* callus cultures. In HPLC analysis, all samples didn't show vinblastine sulphate peak, but the most promising point in this study is the existence of different alkaloid compounds in the extracts of several treatments which need extensive chemical studies to know the type of compounds and their biological activities.

Real time quantitative RT-PCR using SYBR Green I assay was used to analyze the changes in expression of the three of *C. roseus* genes (*Str1- tdc* and *cyp72A1*) in response to different media additives (different concentrations of sucrose, banzyl adenine and Jasmonic Acid). *Cyp72A1* showed maximum folding of gene expression (4.2) between treated and untreated callus under BA (0.2 mg/L benzyl adenine) treatment. This manifesteted the influence of BA in up-regulating this gene. *Str1* gene under Ja2 treatment showed minimum folding (0.3) between treated and untreated callus. The remaining genes represented comparable expression in all treatments. *Str1* gene was up-regulating in all treatments except 4 % sucrose treatment (0.7) and as mentioned before Ja2 treatment (0.3), and about *tdc* gene, it was up-regulated all treatments except 4% sucrose (0.9), while *cyp72A1* gene was up-regulated in all treatments. The results showed that differential gene expression can be detected unequivocally by real time PCR with SYBR Green I assay. It also demonstrated the sensitivity of the assay and its ability to detect subtle changes in gene expression.

Key Words: *Catharanthus roseus* (L.) G. Don, Tiusse culture, alkaloids, benzyl adenine, Jasmonic Acid, RT-PCR, up-regulating, *Str1- tdc* and *cyp72A1* genes

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Approval Sheet

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