MOLECULAR GENETIC STUDIES ON THE PRODUCTION OF PHARMACEUTICALLY IMPORTANT SECONDARY METABOLITES IN SOME MEDICINAL PLANTS

By

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

Mona Mohammed Moghazee Ali Draz. Genetic Studies on Production of some Secondary Metabolites in Medicinal Plants. Unpublished Ph.D. Thesis, Ain-Shams University, Faculty of Agriculture, Department of Genetics, 2018.

The Madagascar periwinkle *Catharanthus roseus* (L.) G. Don (*Apocynaceae*) produces a widespread range of monoterpenoid indole alkaloids (MIAs). Approximately of these secondary metabolites possess therapeutically value. Dimeric MIAs like vincristine and vinblastine that are powerful anti-cancer drugs in widespread use in cancer chemotherapy. Tissue culture of *C. roseus* has been sources of medicinally important MIAs that have suffered from low productivity. Tissue culture technique used to increase these active ingredient components

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, this 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 hours lights and 8 hours darks at 22-25°C. All culture media used in this study were adjusted to pH= 5.6 - 5.8 before solidification with 0.8% Agar.

In this study, we have investigated the effect of yeast extract concentration of 0.4mg/L for three periods (2, 4, and 6 hour) to determine their influence on alkaloid formation in *C. roseus* callus cultures. Addition investigated the effect of transient reporter gene (GUS gene) that presents in *Agrobacterium* cloned into vector pCAMBIA1 1302 of optimal dentistry (OD_{600}.). the vector transformed into *Agrobacterium* at different concentration.

Results demonstrated that OD_{600} = (0.1, 0.4 and 0.8) *Agrobacterium* strain balances the need for maximum delivery of gene construct without causing tissue necrosis and cell death.

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 (*str-dat* and *wrky1*) in response to different media additives (different periods of yeast extract). The influence of YE in up-regulating of these genes; *str1* showed maximum folding of gene expression (9x) between treated and untreated callus under YE1 (0.4 mg/L YE for 2h) treatment while *dat* gene was up-regulated in YE1 (0.4 mg/L YE for 4h) treatment (12x) and *wrky1* showed maximum folding of gene expression (60x) between treated and untreated callus under YE2 (0.4 mg/L YE for 6h) treatment, The remaining genes represented comparable expression in all treatments. The results showed that differential gene expression can be detected 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, Yeast extract, *Agroinfiltration*, RT-PCR, up-regulating, *str1-dat* and *wrky1* genes

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LIST OF ABBREVIATIONS

C. roseus	*Catharanthusroseus (L.) G. Don*
MS medium	Murashige and skoog medium
Kin	Kinetin
JA	Jasmonic Acid
2,4 D	2, 4-dichorophenoxyacetic acid
BA	Benzyl adenine
NAA	α-Naphthaleneacetic Acid
Try.	Tryptophan
HPLC	High performance liquid chromatography
str	Strictosidine synthase
dat	Deacetylvindoline O-acetyltransferase
wrky	*C.roseus* (WRKY1) mRNA, complete cds Transcription Factor
ZCT	zinc-finger Catharanthus transcription factor
ORCA	octadecanoid-responsive Catharanthus AP2-domain protein
BPF-1	Catharanthusroseus box P-binding factor 1 homologue
AP2/ERF	APETALA2/ethylene responsive factor
GUS	*β*-glucuronidases
X-glu	5-Bromo-4-chloro-3-indolylglucuronide
TIAs	terpenoidindole alkaloids
MIA	Monoterpeneindole alkaloid
MS	Molecular Size
PCR	Polymerase Chain Reaction
RT-PCR	Reverse transcriptase PCR
QPCR	Quantitative Polymerase Chain Reaction
TBE	Tris boric EDTA
EtBr	Ethidium bromide
cDNA	complementary DNA

TBE	Tris boric EDTA
EtBr	Ethidium bromide
A.tumefaciens	*agrobacterium tumefaciens*
YE	yeast elicitor or yeast extract
GBF	G-box-binding factor
GA	gibberellic acid
ET	Ethylene
GC-MC	Gas chromatography–mass spectrometry
DW	Dry Weight
OD.	Optical density