

**GENETIC STUDIES ON PRODUCTION OF SOME
SECONDARY METABOLITES IN MEDICINAL
PLANTS**

By

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صفحة الموافقة على الرسالة

دراسات وراثية على إنتاج بعض المركبات الثانوية في النباتات الطبية

رسالة مقدمة من

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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 μ M and 100 μ M) 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, benzyl 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 manifested 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, Tissue 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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TABLE OF CONTENTS

I. INTRODUCTION	Error! Bookmark not defined.
II. REVIEW OF LITERATURE	4
1 The value of plant natural products	4
2 Production of secondary metabolites from medicinal plants	6
3 Usefulness of the <i>In Vitro</i> plant cell system-based technology	8
4 Approaches to increase secondary metabolite productivity of cultured plant cells	9
4.1 Optimization of culture conditions and media components	9
4.2 Addition of precursors	Error! Bookmark not defined.
4.3 Use of chemical elicitor	Error! Bookmark not defined.
4.4 Biotransformation	Error! Bookmark not defined.
5 Alkaloid biosynthesis in plants	Error! Bookmark not defined.
6 Madagascar periwinkle (<i>Catharanthus roseus</i> L.): A biological source for pharmaceutically and economically important compounds	16
6.1 Biosynthesis of <i>Catharanthus</i> alkaloids	17
6.2 Molecular basis of <i>Catharanthus</i> alkaloid biosynthesis	19
6.3 Production options of <i>Catharanthus</i> alkaloids	22
6.4 Diversity of tissue culture of <i>Catharanthus rosues</i> .	Error! Bookmark not defined.
7 Polymerase chain reaction	26
7.1 Reverse transcription-polymerase chain reaction	26
7.2 Real time (quantitative) polymerase chain reaction (qPCR)	27
III. MATERIALS AND MATHODS	Error! Bookmark not defined.
1 Materials	Error! Bookmark not defined.
1.1 Plant Materials	Error! Bookmark not defined.
2 Methods	Error! Bookmark not defined.
3.1 Preparation of Plant Materials	Error! Bookmark not defined.
2.2 Culture conditions	Error! Bookmark not defined.

2.3 Callus initiation and treatments	Error! Bookmark not defined.
2.4 Measurement of Dry Weight Percentage of Fresh Cell.....	32
2.5 Alkaloid extraction and quantification	33
2.5.1 Chemicals	۳۳
2.5.2 Equipment.....	۳۳
2.5.3 Samples extraction and preparation.....	۳۴
2.5.4 HPLC conditions	34
2.6 DNA extraction.	35
2.6.1 Quality check and quantitation of DNA:	3۵
2.7 Agarose gel electrophoresis.....	۳۶
2.7.1 Preparation of agarose gel	۳۶
2.7.2 Loading of DNA on gel	36
2.7.3 Electrophoresis of the gel	36
2.7.4 Gel examination.....	36
2.8 Polymerase Chain Reaction (PCR)	37
2.8.1 Template DNA	37
2.8.2 Primer design.....	37
2.8۳. Primer concentration:	37
2.8.4 Checking amplification and specificity of primers.....	38
2.8.۵. PCR Reaction	38
2.8.6 Optimization of PCR conditions	39
2.9 Preparation of DEPC water	39
2.10 RNA extraction.....	40
2.10.1 DNase treatment	40
2.10.2 Electrophoresis of RNA	41
2.11 Two-step real-time quantitative RT-PCR.....	42
2.11.1 cDNA synthesis	Error! Bookmark not defined.

2.11.2 Real-time qPCR.....	42
2.11.3 Data analysis.....	42
III. RESULTS AND DISCUSSION	٤٥
١ Establishment of tissue culture system in Egyptian <i>Catharanthus roseus</i>	45
١.1 Aseptic seed germination	47
1.2 Selecting explant material	48
1.3 Optimization of culture conditions and media components	50
1.3.1 Effect of different sucrose concentrations on <i>C. roseus</i> callus growth	Error! Bookmark not defined.
1.3.2 Effect of hormone balance on <i>C. roseus</i> callus growth.....	52
2 Treatments to increase <i>Catharanthus</i> alkaloids productivity.....	54
2.1 sucrose treatments.....	55
2.2 Benzyl adenine treatments.....	55
2.3 Jasmonic acid treatments	Error! Bookmark not defined.
3. HPLC analysis	57
4. Molecular analysis.....	60
4.1 Primer design and specificity	60
4.2 Optimization of PCR conditions	Error! Bookmark not defined.
4.3 Selection of the endogenous reference gene.....	٦٠
4.4 Determination of amplification efficiency	٦٣
4.5 Quantification of gene expression	٦٤
4.6 Regulation of <i>str1</i> gene expression under different treatments.....	٦٦
4.7 Regulation of <i>tdc</i> gene expression under different treatments ...	Error! Bookmark not defined.
4.8 Regulation of <i>cyp72A1</i> gene expression under different treatments	Error! Bookmark not defined.
IV. SUMMARY	٧٤
V. References	٧٩

LIST OF TABLES

Table (1): Primer sequences of target genes and endogenous genes used for real-time PCR.....	38
Table (2): Effect of sucrose concentrations on <i>C.roseus</i> callus.....	51
Table (3): Effect of different combinations of plant growth regulators on <i>C. roseus</i> callus growth.....	53
Table (4): C _T values of three target genes and <i>CrActin</i> for treated and control of <i>Catharanthus roseus</i> calli. Each C _T value represents mean of three replicates.....	63
Table (5): $\Delta\Delta C_T$ values and folding levels of <i>strI</i> gene for treated and control calli of <i>c. roseus</i>	Error! Bookmark not defined.
Table (6): The level of <i>tdc</i> gene expression between treated and untreated (control) of <i>c. roseus</i> determined by the comparative $\Delta\Delta C_T$ method	Error! Bookmark not defined.
Table (7): The level of <i>cyp72A1</i> gene expression between treated and untreated (control) of <i>c. roseus</i> determined by the comparative $\Delta\Delta C_T$ method ...	Error! Bookmark not defined.

LIST OF FIGURES

Figure (1): Terpenoid indole alkaloid biosynthetic pathway in <i>C. roseus</i>	19
Figure (2): A typical amplification plot obtained with a real-time PCR assay	28
Figure (3): <i>catharanthus roseus</i> varieties: (a) var. albus (b) var. roseus (c) var. ocellatus	47
Figure (4): Sterilized seedlings of Egyptian <i>Catharanthus roseus</i> grown on half-strength of solid basal MS medium.....	48
Figure (5): Explant types used (a) hypocotyl (b) cotyledons and (c) mature leaf sections in two different types of fragmentation.....	49
Figure (6): Healthy calli with good size, shape and color produced using transversal leaf sections as explants.....	50
Figure (7): Effect of different sucrose concentrations on colour, size and the degree of compaction of the <i>C. roseus</i> callus.	51
Figure (8): Effect of different combinations (M1 for 1mg/L 2,4D + 0.1 mg/L kin and M2 for 1mg/L 2,4D + 0.1 mg/L BA) of plant growth regulators on callus growth.....	53
Figure (9): Effect of three concentrations of sucrose (S1, S2 and S3) on callus performance comparing with control.	55
Figure (10): Effect of three concentrations of benzyl adenine (BA1, BA2 and BA3) on callus performance	56
Figure (11): Effect of two concentrations of jasmonic acid (Ja1 and Ja2) on callus performance	57
Figure (12): The HPLC chromatograms of standard vinblastine sulphate.	57
Figure (13): The HPLC chromatograms of extracted alkaloids from sucrose treated calli comparing with control	Error!
	Bookmark not defined.

- Figure (14): The HPLC chromatograms of extracted alkaloids from benzyl adenine treated calli comparing with control59
- Figure (15): The HPLC chromatograms of extracted alkaloids from jasmonic acid treated calli comparing with control.. **Error! Bookmark not defined.**
- Figure (16): A representative view of the amplification plot generated to determine the expression of candidate endogenous reference gene (*CrActin*).....62
- Figure (17): qPCR reaction products for the target genes (*str1*, *tdc* and *cyp72A1*) and the reference gene (*CrActin*) under for treated and untreated calli65
- Figure (18) The folding levels of *str1* gene between treated and untreated (control) of *c. roseus* **Error! Bookmark not defined.**
- Figure (19): The folding levels of *tdc* gene expression between treated and untreated (control) of *c. roseus*.**Error! Bookmark not defined.**
- Figure (20): The folding levels of *cyp72A1* gene expression between treated and untreated (control) of *c. roseus*. **Error! Bookmark not defined.**
- Figure (21): The folding level of gene expression for three target genes *str1*, *tdc* and *cyp72A1* between treated and untreated (control) of *c. roseus* under sucrose treatments. **Error! Bookmark not defined.**
- Figure (22): The folding level of gene expression for three target genes *str1*, *tdc* and *cyp72A1* between treated and untreated (control) of *c. roseus* under BA treatments. **Error! Bookmark not defined.**
- Figure (23): The folding level of gene expression for three target genes *str1*, *tdc* and *cyp72A1* between treated and untreated (control) of *c. roseus* under ja treatments. **Error! Bookmark not defined.**