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Influence of Some Biotic and Abiotic Elicitors on Production of Indole Alkaloids in Callus and Cell Cultures of *Catharanthus roseus*

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By

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Title of the M. Sc. Thesis

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Dedication

To my parents

And my brothers

For their deep concern,

Support, endless patience

And love.

DECLARATION

This thesis has not been previously submitted for a degree at this or at any other university and is the original work of the writer

Eman Abdallah Mohamed Ali

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Abstract

Influence of Some Biotic and Abiotic Elicitors on Production of Indole Alkaloids in Callus and Cell Cultures of *Catharanthus roseus*

1. Abstract

Catharanthus roseus L. (G) Don. (Apocynaceae) is an important medicinal plant as it is the sole source of vincristine and vinblastine that are used against a variety of cancers. The objective of the present thesis was to maximize the indole alkaloid production from *C. roseus* suspension cultures by optimizing the cultural conditions. Mycofloral surveys of phylloplane and stemplane of *C. roseus* allover three months revealed 116 fungal species (33 from phylloplane and 83 from stemplane) were isolated / 3 months. *Alternaria alternat*a (the cause of leaf spots in *C. roseus*) was the most dominant in both localities and selected for elicitation in suspension culture. The optimum growth media for hypocotyls derived calli was MS_3 [containing 2,4-D and BA (0.5:1 mg l^{-1})] while MS_4 [containing 2,4-D and BA (0.5:0.5 mg l^{-1})] was the optimum of cotyledons and leaves derived calli. The optimum production medium for indole alkaloid induction from hypocotyls and cotyledons suspension culturres was MS_{4d} (containing 25 % of the original 2,4-D concentration and 4 % instead of 3 % sucrose, while the unmodified MS_{6a} was optimum for indole alkaloid production by *C. roseus* leaves suspension cultures. The three stress factors (*A. alternata* elicitor, $VaSO_4$ and KCl) enhanced alkaloid production by *C. roseus* suspension cultures either tested individually or in combinations. Among the 19 experimental combinations MS_{4d} + mixture (1) of stress factors (100 mg / g dry cells *A. alternata* elicitor, 2.75 mg / g dry cells $VaSO_4$ and 275 mg / g dry cells KCl) was the optimum production media and resulted in promising production of indole alkaloids from hypocotyls suspension cultures of *C. roseus*. HPLC analysis indicated the presence of both extracellular and intracellular vincristine and vinblastine in the suspension cultures of *C. roseus*. Vincristine quantity was much higher than vinblastine either in extracellular or intracellular samples.

Key words: *C. roseus*, Indole alkaloids, biotic elicitors, abiotic elicitors, *A. alternata*, vincristine, vinblastine.

Introduction

2. Introduction

The tropical plant Madagascar Periwinkle (*Catharanthus roseus*), which is a member of Apocynaceae family, is a rich source of the indole alkaloids of high medicinal and economic value such as anticancer indole alkaloids vincristine and vinblastine (Moreno *et al*., 1995), which complexity makes it impossible to synthesize in laboratory (Costa *et al.,* 2008). It is also a source of other medicinally important indole alkaloids such as ajmalicine used as antihypertensive compound, serpentine with sedative activity (Sottomayor and Ros Barceló, 2005 and Van der Heijden *et al.,* 2004), catharanthine, and vindoline. Moreover, the ethanolic extract of leaf has significant hypoglycemic activities, rendering it as a good antidiabetic drug (Kar *et al*., 2003). However, alkaloids occur in the plant in salt form. Boiling water was used to extract alkaloids from leaves and stems and these aqueous extracts inhibit acethylcolinestherase, So *C. roseus* opens another perspective for the medicinal use of this plant (Pereira *et al.,* 2009).

The leaves of this species being still today, the only source of vinblastine and vincristine (Costa *et al.,* 2008), while the roots of the plant accumulate ajmalicine and serpentine (Shukla *et al*., 2006).

Vincristine suffers from the disadvantage of very low yield from the source material and so is prohibitively expensive. Vinblastine and vincristine accumulate in trace amounts in leaves and are formed from the oxidative coupling of catharanthine and vindoline (Van der Heijden *et al*., 2004). Vindoline is abundant in *C. roseus* (0.2% as a dry weight basis) and less expensive compared to vinblastine and catharanthine.

The extraction of chemical plant products from intact plants has several inherent problems, including seasonal variations, pests, diseases and inconsistent product quality and yield (Kargi and Potts 1991). Attempts to produce these alkaloids industrially by cell suspension cultures are unsuccessful due to many biological and technological limitations (Kargi, 1988; Verpoorte *et al*., 1997). One of these limitations is the low yield of these compounds in the cell cultures due to lack of some enzymes in the pathway leading to the formation of the necessary intermediates