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Effect of fungal elicitation on growth and metabolite production in callus of few medicinal plants

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ABSTRACT

With the developing use of herbal drugs, the need of plants containing secondary metabolites which have medicinal value has increased. Since the intact plant contains low concentrations of active compound, plant callus cultures have employed as an alternative to produce large amounts of these secondary metabolites. Moreover using a bioelicitor the secondary metabolite production can be increased. The objective of this study was to develop a rapid system for the enhanced production of Guggulsterones from *Commiphora wightii*, Zingiberene from *Zingiber officinale* and Falcarinol from *Daucus carrota* *Aspergillus niger* cell extract was used as an elicitor to stimulate the production of secondary metabolite. But inverse results were obtained showing a considerable decrease in Secondary metabolites and growth as well. This is supposed from the results that the mycotoxins may have hindered the metabolite production or probably the biosynthetic pathway was not affected. The same experiment is repeated with cell suspension cultures.

Key words: fungal elicitation *Commiphora wightii, Zingiber officinale, Daucus carrota* *Aspergillus niger.*

INTRODUCTION

The accumulation of secondary metabolites in plants is part of the defense response against pathogenic attack, which is triggered and activated by elicitors, the signal compound of plant defense responses. Therefore, the treatment of plant cells with elicitors has been one of the most useful strategies to enhance secondary metabolites production in plant cell cultures and is recently finding commercial application (Zang et al., 2000). Elicitors are microbe derived molecules which can enhance secondary metabolite production in cultured cells (Dicosmo

and Misawa, 1985). They trigger the increased production of pigments, flavones, phytoalexins and other defense related compounds. Elicitors from fungal origin have been widely employed to increase natural product formation in plant cell cultures and this strategy has been effective in stimulating the production of many chemical classes of secondary metabolites such as terpenoids, coumarin derivatives, alkaloids and flavonoids.

Recently there is a report on increase in production of andrographolide by *Aspergillus niger* and *Penicillium expansum* elicitors in cell suspension culture of *Andrographis paniculata* (Vakil and Mendhulkar, 2013). An oligosaccharin, originating from fungal cell wall is known to induce a number of specific biochemichal changes associated with resistance leading to the synthesis of phytoalexins, lignin and ethylene formation (Ryan, 1988). Since then it has been demonstrated that yeast cell wall preparations, bacterial antibiotics, plant cell wall-derived oligouronides, fungal cell wall derived chitosan, N-acetyl chitohepatose and wounding lead to the rapid synthesis of endogenous jasmonic acid (Muller, 1997).

MATERIALS AND METHODS

Callus cultures of *Commiphora wightii*, *Zingiber officinalis and Daucus carrota* were grown on selected modified Murashige and Skoog (MS) medium. MS modified media [MS-stocks $ABD_{1/2}$ strength (950 mg/l KNO_3, 825 mg/l NH_4NO_3 and 220mg/l $CaCl_2.2H_2O$)] was prepared by mixing salts (in the form of stock solution or weighing fresh every time) and other ingredient in required volume. The media were solidified with 0.8% agar; pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20-25 minutes under 1.05 kg/cm^2 pressures. The callus cultures were initiated on modified Murashige and Skoog (Murashige and Skoog, 1962) medium (950 mg/l KNO_3, 825 mg/l NH_4NO_3 and 220mg/l $CaCl_2.2H_2O$) containing 2, 4-D (0. 5mg/l) and kinetin (0.1 mg/l) (Kumar et al., 2003). Medium was poured in 100 or 250 ml conical flasks contained 20 ml, 35 or 50 ml medium, respectively. Culture were kept vertical to allow cooling and gelling of the medium. Oven dried glassware were used for all experiments. All the cultures were incubated under white fluorescent light 75 µ mol $s^{-1}m^{-2}$ photon flux, 16 hr photoperiod at 26^{0}C temperature and 60% relative humidity. The cultures were monitored daily and the readings were recorded after 4 weeks. The callus cultures were dried and extracted in methanol and HPLC analysis of callus cultures were done and pure or analytical grade chemicals were used throughout the course of study. Preparation of fungal elicitor (Staniszewska *et al.*,2003)

*Aspergillus niger*was grown in 250 ml flasks containing nutrient broth. The flasks were incubated at room temperature under static conditions. At stationary phase, after 21 days, the flasks were autoclaved and the fungal mat separated from the culture medium / filtrate. The culture filtrate was filtered through Whatman No.1 filter paper and made up to a known volume, and autoclaved and stored at 4°C and designed as culture media filtrate.

The fungal mat was washed several times with distilled H2O and an aqueous extract was prepared by homogenizing in a mortar and pestle using acid washed neutralized sand. This extract was filtered through muslin cloth or centrifuged and the clear supernatant was taken. The supernatant is made up to a known volume, autoclaved and stored at 4°C and designated as mat extract. Suspension of *A. niger* cell extract, OD600 = 1.2 (15 ml/l) was added to MS medium. Elicitor was added to MS medium directly before planting cell suspension culture to a new flask.
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RESULTS AND DISCUSSION

Guggulsterones from *Commiphora wightii*, Zingiberene from *Zingiber officinale* (Ernest J.V. Cafino et.al, 2015)and Falcarinol from *Daucus carrota* (Eva M P Wenzig et.al, 2009) were targeted to increase by elicitation with *Aspergillus niger* cell extract. But a considerable decrease in secondary metabolites and growth was observed (Table 1). According to already known fact if the growth retards the secondary metabolite are said to be increased due to the stress created. But here in this case callus from all three medicinal plants didn't showed an increase in growth

Table 1: Effect of elicitor on growth and metabolite production in plants

S. No	Name of Plant	Elicitor (*Aspergillus niger*)	Metabolite present	Metabolite content**(µg/g)**	Dry weight of callus
1.	*Commiphora wightii*	Not added	Guggulsterone	8.1	2.1±0.06
		Added		2.3	1.1±0.05
2.	*Zingiber officinale*	Not added	Zingiberene	2.7	4.1±0.02
		Added		0.9	2.3±0.01
3.	*Daucus carrota*	Not added	Falcarinol	3.5	5.1±0.04
		Added		1.6	3.2±0.05

nor the bioactive molecules targeted increased. Probably the mycotoxins may have hindered the production or the biosynthetic pathway was not affected. The same experiment is repeated with cell suspension cultures.

Conflicts of interest: The authors stated that no conflicts of interest.

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