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A combination of elicitor and precursor enhances psoralen production in *Psoralea corylifolia* Linn. suspension cultures

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ABSTRACT

Psoralen is a pharmaceutically important furanocoumarin produced by *Psoralea corylifolia* Linn. A strategy for the enhanced production of psoralen has been developed using suspension cultures of *P. corylifolia* through combined elicitation and elicitor and precursor feeding. Methyl jasmonate (MeJA) and salicylic acid (SA) were used as elicitors with one of the psoralen biosynthesis pathway precursor *trans*-Cinnamic acid (CA). Either MeJA (or) SA individually at 100 µM enhanced psoralen production higher than control (13.6 and 10.2 folds respectively), however, administration of these two elicitors together did not result in enhancement of psoralen production. While, either MeJA or SA in combination with 16.8 mM CA exhibited positive synergetic effect on psoralen production. A combination of 100 µM MeJA with 16.8 mM CA exhibited the highest psoralen production (24.9 folds higher than control) compared with the other elicitor combinations used in the study. Results of the present study, demonstrate that, MeJA is an influential elicitor for enhancing psoralen production and in combination with precursor (CA) proved promising for higher psoralen yield, and this could be the effective strategy.

1. Introduction

Psoralea corylifolia Linn., is a herbaceous plant belongs to Fabaceae. Since ancient times the plant has been suggested for the treatment of various skin conditions such as leucoderma, leprosy, psoriasis and inflammatory diseases of the skin. Besides these, the plant also possesses antitumor, immunomodulatory, anti-inflammatory and anti-proliferative activities (Chopra et al., 2013). *P. corylifolia* is known for its source of psoralen, distributed throughout the plant (Baskaran et al., 2008). Psoralen, a linear furanocoumarin is the main bioactive compound associated with these biological effects of *P. corylifolia,* that is synthesized via phenylpropanoid pathway (Bourgaud et al., 2014). The rising demand for psoralen and its low productivity in natural habitat has prompted considerable efforts to find new sources for the enhanced production. Plant cell cultures are one of the promising alternative sources for the production of secondary metabolites like psoralen.

Secondary metabolites are the valuable biological compounds produced by plants in response to stress, which also have biological activities. Secondary metabolites are responsible for the adaptation of plants to environmental stimuli or may serve as protection against being eaten by herbivores or being infected by microbial pathogens (Udomsuk et al., 2011). Callus cultures challenged with elicitors or precursors could up regulate the secondary metabolite biosynthesis in plant cell cultures (Rao and Ravishankar, 2002). Sometimes in vitro derived cell cultures (callus) may fail to synthesize the secondary metabolites, which might be attributed to lack of environmental stimuli or an inadequate expression of developmentally regulated biosynthetic genes. However, exogenous supplementation of precursors to the medium induces a subset of genes associated with secondary metabolite biosynthesis, modulate their expression and promote the accumulation of desired metabolites. Cultures of *Rhodiola rosea* failed to produce cinnamyl glycosides in callus until the medium was supplemented with cinnamyl alcohol (Gyorgy et al., 2004). Cinnamic acid (CA) is one of the precursors involved in the synthesis of psoralen. Mohammadparast et al. (2014), demonstrated that, addition of CA to the medium of *P. corylifolia* callus cultures increase the psoralen content, opening new ways to enhance the bioactive compound. Methyl jasmonate (MeJA) and salicylic acid (SA) have been found to be effective elicitors when added to the culture medium for the production of secondary metabolites (Guo et al., 2015; Wang et al., 2015). It has been demonstrated that, in the suspension cultures elicited with either MeJA or SA increase the generation of reactive oxygen species (ROS), which in turn react

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with cellular components, thereby causing an immediate plant defense response (Chen et al., 1993; Suhita et al., 2004). It has also been demonstrated that, combining the elicitor and precursor commonly up regulate the biosynthesis of secondary metabolites and also directs to extend the competency of cell cultures (Rahpeyma et al., 2015; Hidalgo et al., 2017). Keeping in view of these facts, during the present study the effect of elicitor-precursor combination on psoralen production has been investigated in *P. corylifolia* cell suspensions.

2. Materials and methods

2.1. Chemicals

Elicitors such as MeJA, SA, precursor CA and psoralen were procured from Sigma-Aldrich, USA. High performance liquid chromatography (HPLC) grade solvents (Hi-Media, India; SRL, India) were used, and the other chemicals used in the study were of analytical grade (HiMedia, India).

2.2. Suspension cultures

Cell suspension cultures were established from one month old friable leaf callus of *P. corylifolia*. Leaf callus was induced on solid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing either 9.049 μM 2,4-dichlorophenoxyacetic acid (2,4-D) or 0.828 μM picloram. Callus (1 g) of was inoculated to 50 ml liquid medium containing same growth regulators (used for callus induction) in 250 ml conical flask and cultures were incubated for 24 day period and maintained in dark at 25 °C in a rotary shaker incubator at 110 rpm. Elicitors were added on the day of inoculation and the cells were harvested at regular time intervals each at 6, 12, 18 and 24 days. Cells were collected by filtration for the measurement of cell growth and quantification of psoralen.

2.3. Elicitor preparation

Two elicitors were used in the present study. SA was initially dissolved in 1 N NaOH, made up to final concentration with sterile distilled water and filter sterilized before use at concentrations ranging from 50 μM, 100 μM and 200 μM. While, MeJA was initially dissolved in methanol, made up to final concentration with sterile distilled water and filter sterilized before use at concentrations ranging from 50 μM, 100 μM and 200 μM. In control cultures elicitors were substituted with sterile distilled water. Similarly precursor CA was used at 16.8 mM concentration (Mohammadparast et al., 2014). CA was initially dissolved in methanol final concentration (of 16.8 mM) was made up with sterile distilled water and filter sterilized before adding to the media. Individual elicitor concentrations were optimized for the production of psoralen in *P. corylifolia* cell suspension cultures and used for combined elicitation, to study the synergistic interaction between elicitors and precursor combinations. The optimized elicitor concentrations and their respective combinations used for the investigations were, 100 μM MeJA + 100 μM SA, 100 μM MeJA + 16.8 mM CA and 100 μM SA + 16.8 mM CA.

2.4. Analysis of cell growth

At the end of each culture period cells were separated from the media by passing them through Whatman No. 1 filter paper. Fresh cell weight (FCW) of each biomass was determined after washing with distilled water, excess water was blotted away placing between folds of Whatman filter paper. Cell growth was determined as dry cell weight (DCW) of cell mass and was recorded after drying cell mass in a hot air oven at 45 ± 2 °C till it attains constant weight. To reduce the differences in cell growth caused by the variation in inoculum size, growth index was used. Cell growth was determined as, $growth\ index = \frac{Wt\ final - Wt\ initial}{Wt\ initial}$ where, Wt_{final} is weight of inoculum at the end of culture period, and $Wt_{initial}$ is weight of inoculum at 0 day.

2.5. Measurement of medium conductivity and pH

Cells were collected by filtration and the filtrate was used for measurement of medium conductivity using conductivity meter (Model 304, Systronics, India). Media pH were determined using pH meter (Model LI 120 pH meter, ELICO India).

2.6. Extraction of psoralen

Extraction and quantification of psoralen was carried out as described by Baskaran and Jayabalan (2008). Dried cells (1 g) were ground into fine powder using liquid nitrogen and soaked overnight in 10 ml methanol. The residue was recovered by passing the mixture through Whatman No.1 filter paper. Residue was dissolved in 5 ml methanol, centrifuged at 1200 rpm for 5 min. Supernatant was filtered through 0.45 μm nylon filter, was injected to HPLC to quantify psoralen.

2.7. HPLC analysis for psoralen

Psoralen was quantified by HPLC using Agilent 1260 infinity HPLC unit equipped with diode array detector (DAD), separation was carried out with C18 column (Zorbax Eclipse plus C18, 4.6 × 250 mm, 5 μM) at 20 °C, methanol, water (50:50) used as mobile phase with flow rate 0.8 ml /min, psoralen was detected at 254 nm. Obtained HPLC peaks were correlated with psoralen standard prepared in HPLC grade methanol.

2.8. Statistical analysis

All the experiments were performed in triplicates under the same conditions individually. Data were expressed as Mean ± SE. Significant difference between the treatments were analyzed by one-way ANOVA using Tukey-Kramer (HSD) principle range test at $P \leq 0.05$ (Assad et al., 2014). Graphs were drawn using Micro Soft Excel programme.

3. Results and discussion

The present study was carried out to study the influence of elicitors MeJA, SA and precursor CA either individually or in combination on biomass and psoralen production in cell suspension cultures of *P. corylifolia*.

3.1. Analysis of cell growth

Cell growth was monitored in terms of DCW and results are depicted in Fig. 1. The treatment of cells with MeJA, SA and precursor CA (Fig. 1a–c, respectively) showed decrease in cell growth throughout the culture regimen compared with untreated control set of cultures. Cell growth was observed to be decreased with the increasing concentration of MeJA and SA above 50 μM. As far as MeJA is concerned maximum cell growth was obtained on 12th day with 50 μM (4.47 ± 0.12 g/l DCW), 100 μM (3.57 ± 0.0882 g/l DCW) and 200 μM MeJA (2.6 ± 0.351 g/l DCW) that were 0.95, 1.3 and 1.79 times less compared with control cultures. Similarly with SA maximum cell growth was obtained on 24th day at 50 μM (5.73 ± 0.0933 g/l), 100 μM (4.43 ± 0.08 g/l DCW) and 200μM (4.13 ± 0.06 g/l) that were also observed to be 0.97, 1.33 and 1.4 times lesser than the control. While elicitors combination 100 μM MeJA + 100 μM SA and individual elicitor-precursor combinations i.e., 100 μM MeJA + 16.8 mM CA and 100 μM SA + 16.8 mM CA also resulted in significant decrease in cell