Saudi Journal of Biological Sciences (2014) 21, 499–504

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Biotic elicitor enhanced production of psoralen in suspension cultures of *Psoralea corylifolia* L.

Syed Abrar Ahmed [a], Mirza Mushtaq Vaseem Baig [b,*]

[a] *Department of Botany, Maharashtra College of Arts, Science and Commerce, Mumbai Central (E), Mumbai, 400008 M.S., India*
[b] *Department of Botany and Department of Biotechnology, Yeshwant Mahavidyalaya, Nanded 431602 M.S., India*

Received 4 October 2013; revised 19 December 2013; accepted 25 December 2013
Available online 8 January 2014

KEYWORDS

Elicitation;
Psoralen;
Psoralea corylifolia L.;
Suspension cultures

Abstract Cell cultures of *Psoralea corylifolia* L. were established from the leaf disk derived callus. The effect of different biotic elicitors prepared from the fungal extract (*Aspergillus niger* and *Penicillium notatum*), yeast extract and chitosan with different concentrations was studied. The increased synthesis of psoralen in 16-day old cell cultures under 16 h of light and 8 h of dark period was studied. Elicitation of psoralen in *A. niger* elicitor treated cells was found 9-fold higher over control cells. Treating the cells with *P. notatum*, yeast extract and chitosan elicitors lead to four to seven-fold higher psoralen accumulation over control cells. The extract of *A. niger* at 1.0% v/v increased the significant accumulation of psoralen (9850 μg/g DCW) in the cultured cells. Our study clearly shows that all the elicitors had the potential to increase the accumulation of psoralen but the *A. niger* elicitor at 1.0% v/v induced maximum accumulation.

© 2014 Production and hosting by Elsevier B.V. on behalf of King Saud University.

1. Introduction

Plants are the prime source of medicinally important compounds. Many plant products are used as pharmaceuticals, pigments, herbicides, etc. Plant cell culture has been used for the production of various valuable phytochemicals. *Psoralea corylifolia* L. is an important medicinal plant found in the tropical and subtropical regions of the world. It synthesizes diverse phenylpropanoids such as furanocoumarins, isoflavonoids etc. (Boardley et al., 1986). Psoralen is the furanocoumarin and commercially important for having broad range of pharmacological activities such as photosensitizing, photobiological and phototherapeutic properties (Frank et al., 1998). Psoralen has been used for the photochemotherapy of vitiligo and skin diseases such as psoriasis, mycosis fungoides and eczema (Khushboo et al., 2010; Ozkan et al., 2012). It also shows antitumor (Szliszka et al., 2011), antibacterial (Chanda et al., 2011) and antifungal properties (Srinivasan and Sarada, 2012). Plant cell culture offers uniform secondary product synthesis by overcoming the effect of unforeseen climatic conditions and diseases in field grown plants. Elicitors are one of the important factors that can act as a switch for increasing the yield of secondary metabolites of useful bioactive compounds in plant cell cultures (Gaid et al., 2011; Wiktorowska et al., 2010 and Karwasara et al., 2010). Most of the elicitors used in earlier studies originated from fungal (Karwasara

* Corresponding author. Tel.: +91 2462 269623; fax: +91 2462 268560.
E-mail addresses: sdabrar645@yahoo.com (S.A. Ahmed), mmvbaig@gmail.com (M.M.V. Baig).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2013.12.008
1319-562X © 2014 Production and hosting by Elsevier B.V. on behalf of King Saud University.

et al., 2011 and Taha et al., 2012), bacterial (Seung-Mi Kang et al., 2009) and yeast cell extracts (Kundu et al., 2012 and Sun et al., 2012) or their purified fraction. In addition some abiotic stress agents like heavy metals, UV light, osmotic stress and pH are also known to increase secondary metabolite accumulation in cultured cells. However, there is no way to predict that an elicitor will be effective in a specific cell system on metabolite accumulation. The main aim of the present work was to study the effect of different elicitors prepared from fungi (*Aspergillus niger* and *Penicillium notatum*), yeast and chitosan on psoralen accumulation in cultured cells of *P. corylifolia*.

2. Materials and methods

2.1. Plant material

P. corylifolia L., plants were grown in the greenhouse of botanical garden of Yeshwant Mahavidyalaya, Nanded and were used for experimental purpose. Young leaves from 3 month-old plants were washed thoroughly under running tap water for 10 min and surface sterilized with 0.1 % (w/v) aqueous $HgCl_2$ solution for 2 min. Finally the leaves were washed 4–5 times with sterile distilled water to remove any traces of $HgCl_2$.

2.2. Culture media

The explants were cut into circles of 1 cm diameter, then were transferred on ready-made (Murashige and Skoog, 1962) basal medium (Himedia, Mumbai) supplemented with 1 mg 2,4-dichloro phenoxy acetic acid (2,4-D) l^{-1}, 0.5 mg naphthalene acetic acid (NAA) l^{-1}, 1.5 mg benzylaminopurine (BAP) l^{-1} (Sigma–Aldrich, USA), and 3% w/v sucrose. The medium was solidified with 0.8% agar (Himedia, Mumbai) and the pH of media was adjusted to 5.8 using 0.1 N NaOH or HCl before autoclaving. Twenty ml media was dispensed in each 1.25×15 cm test tubes (Borosil) plugged with nonabsorbent cotton wrapped with muslin cloth. The test tubes containing medium were autoclaved at 1.06 kg cm^{-2} at 121 °C under 15 lbs/sq. ft. pressure for about 20 min.

2.3. Maintenance of culture

The cultures were maintained under fluorescent light with 16 h photoperiod (40 $\mu E\ m^{-2}\ S^{-1}$) by cool daylight fluorescent incandescent tubes (40 W, Philips, Kolkata) at 25 ± 2 °C. Callus cultures thus raised were transferred to fresh medium once in every 3 weeks.

2.4. Cell suspension cultures

Suspension cultures were initiated using 6 g callus as inoculum in a 250 ml conical flask containing 100 ml of the modified MS medium without agar. The cultures were shaken on a rotary shaker at 120 rpm. The pH of the medium was adjusted to 5.8 by 0.1 N NaOH/HCl before autoclaving. Culture medium was autoclaved at 121 °C under 15 lbs/sq. ft. pressure for 20 min.

The cells were collected on the 16th day by filtration and blotted with filter paper to remove excess water and fresh weight was determined. Dry weight (with ~10% moisture) was determined after drying the cells at 60 °C until a constant weight was obtained. All experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml growth medium inoculated with 10 ml cell suspension (16-day old cultures).

2.5. Fungal elicitor preparation

Elicitors were prepared from cultures of *A. niger* (NCIM No.621) and *P. notatatum* (NCIM No.757) received from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. The fungal filaments were grown in 1000 ml conical flasks containing 250 ml of potato dextrose broth for 10 days at room temperature. Fully grown mycelia with spores were homogenized and centrifuged at 4000 rpm and the supernatants were autoclaved for 20 min at 121 °C and used as elicitors (Kim et al., 2011).

2.6. Yeast elicitor

Ten grams of yeast extract was dissolved in 100 ml of double distilled water and ethanol was added up to 80% (v/v) and was kept at 4 °C for 3 days for precipitation. The supernatant was decanted and the precipitate was redissolved in 100 ml double distilled water then autoclaved and was used as elicitor (Diwan and Malpathak, 2011).

2.7. Chitosan

Chitosan, a well-known elicitor for plant cell cultures (Santamaria et al., 2011) was also used to study psoralen accumulation. The stock solution was prepared by dissolving 1 g of crab shell chitosan (Sigma Chemical Company, USA) in 2 ml of glacial acetic acid by adding it drop wise. The final volume was made up to 100 ml by adding double distilled water. The pH of the solution was adjusted to 5.7 with NaOH or HCl before autoclaving (Malerba et al., 2012).

2.8. Elicitation method

The cell cultures of *P. corylifolia* L. were grown in 250 ml Erlenmeyer flasks having 100 ml of growth medium. Different concentrations of elicitor viz. extract of *A. niger*, *P. notatum,* yeast (0.5–3 % v/v) and chitosan (25–300 mg/l) were added on the 16th day to the cultures. Since the stationary phase was found from 16th to 24th day of growth. The elicitors were added during the stationary phase of growth and the cultures were incubated. The optimization of higher psoralen accumulation in cultures varied from 12 h to 96 h incubation and standardized their concentration for maximum metabolite accumulation. After harvesting the cultures, media and cells were separated and the fresh and dry weight was determined after blotting the cells with filter paper to remove excess of water. Dry weight was determined after drying the cells at 60 °C in a hot air oven until a constant weight was obtained. Psoralen was extracted from both spent media and cells separately and analyzed by HPLC. The biomass was expressed in grams Dry Cell Weight per liter (g DCW/l) and the product

Download English Version:

https://daneshyari.com/en/article/4406204

Download Persian Version:

https://daneshyari.com/article/4406204

Daneshyari.com