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Optimization of elicitation condition with Jasmonic Acid, characterization and antimicrobial activity of Psoralen from direct regenerated plants of *Psoralea corylifolia* L

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ABSTRACT

In present study, estimation and characterization of Psoralen were done for Jasmonic Acid treated *in vitro* plants of *Psoralea corylifolia* L. The elicitor Jasmonic Acid at different concentrations (50,100,150, 200 and 250 μM/L) was used along with 6% sucrose and plant growth regulators to enhance psoralen content in leaves and roots of *P. corylifolia*. Shoot tip explant cultured on MSB_5 medium containing 2.0 mg/L BAP showed higher induction and proliferation of multiple shoots with 82.2% of response and 16.3 shoots/explant. *In vitro* rooting was best achieved with 82.6% of response and 18.6 roots/shoot at 0.8 mg/L IAA. JA at 150 μM/L concentration was found to enhance Psoralen content in leaves and roots of *in vitro* regenerated plants of *P. corylifolia*. The presence and structure of psoralen in methanolic extract of leaves and roots were confirmed through HPLC (retention time 21.007 in root, 20.659 in leaf sample), UV (213 nm), FT-IR, and NMR ($C_{11}H_6O_3$). The amount of Psoralen in JA treated leaf and root sample were found to be higher 1.17 mg/mL and 3.93 mg/mL respectively when compared to naturally growing *P. corylifolia* (0.56 mg/mL). The methanolic extract of root sample showed effective antimicrobial activity against tested bacterial and fungal pathogens. The present work provides the possibility of increasing production of psoralen in *in vitro* plants of *P. corylifolia* using Jasmonic Acid as an elicitor.

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1. Introduction

Psoralea corylifolia L. (Fabaceae) is an endangered medicinal plant distributed widely in the tropical and sub-tropical regions of India (Jain, 1994). It is one of the promising medicinal plants which contains a wide array of chemical compounds including psoralen, isopsoralen, bakuchiol, psoralidin, bakuchalcone, bavachinin, flavones, volatile oils, lipids etc. in different parts (Boardley et al., 1986; Chopra et al., 2013). *P. corylifolia* offers immense biomedical applications against several diseases such as leucoderma, leprosy, psoriasis, vitiligo due to the presence of several isoflavonoids and furocoumarins. Pharmacological tests revealed that they have antitumor, antibacterial and antiviral activities. Of the several bioactive compounds, psoralen, is one of the important furocoumarin abundantly available in this plant which is also widely employed as an anticancerous agent against leukemia and other cancer lines (Latha et al., 2000; Oliveira et al., 2006; Wang et al., 2009; Xin et al., 2010).

There are several reports on the medicinal properties and pharmacological activities of each compound isolated from *P. corylifolia*. Hepatoprotective activity by Bakuchiol along with bakuchicin and psoralen against tacrine-induced cytotoxicity in human liver-derived Hep G-2 cells (Uikey et al., 2011). Bakuchiol, psoralen, isopsoralen, psoralidin, coumarin, angelicin, corylifol-A have been reported to exhibit antitumor activity against various cancer cell lines (Kubo et al.,1989; Guo et al., 2003; Wang et al., 2011; Parast et al., 2011; Mar et al., 2001; Song et al., 2013). DNA

Abbreviations: KIN, Kinetin; BAP, 6 Benzyl aminopurine; TDZ, Thidiazurone; NAA, Naphthalene acetic acid; IAA, Indole-3-acetic acid; IBA, Indole-3- butyric acid; μM, micromole; mL, milli litre; HPLC, High Performance Liquid Chromatography; FTIR, Fourier Transform InfraRed; NMR, Nuclear Magnetic Resonance; UV, Ultra Violet

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polymerase inhibition activity by Corylifolin and bakuchiol (Sun et al., 1998), protein kinase inhibition activity by Corylifol-C (Limper et al., 2013), antifungal activity by pyranocoumarin (Srinivasan and Sarada, 2012), antibacterial activity by bakuchiol (Sandra et al., 2002) have also been reported from *P. corylifolia*. Strong antioxidant activity of four compounds (bakuchiol, corylifolin, corylin and psoralicin) isolated from the seeds of *P. corylifolia* has been described (Uikey et al., 2011).

Furocoumarins are natural plant metabolites characterized by a furane moiety fused to benzopyran-2-one. Furocoumarins intercalate in double-stranded DNA, and psoralens are known to cross-link pyrimidine bases under irradiation by [2+ 2] cycloaddition via their 3, 4- and 2, 3-double bonds (Kanne et al., 1982). The position of the urane substitution distinguishes two large groups of compounds, the linear (psoralens) and the angular furocoumarins (angelicin and derivatives) (Murray, 2002). Psoralen, an important furocoumarin is known for its photosensitizing and phototoxic effects and has been used in photo chemotherapy of skin disorders (psoriasis, vitiligo, and mycosis). Due to the complex bioactivity of furocoumarins, its biosynthesis has received continuous attention. Knowledge of the biosynthetic pathway of psoralen may enable to influence its formation in a direct way by metabolic pathway engineering. The biosynthetic pathways to the linear furocoumarin (Psoralen) involved enzymes (and their co-factors) which are as follows 1. DMAPP- umbelliferone dimethylallyl transferase, 2. Marmesin synthase (O_2, cytochrome P450, NADPH) and 3. Psoralen synthase (O_2, Cyt, P450, NADPH) (Croteau et al., 2000). Growing commercial interest in the production of Psoralen for the development of pharmacological agents posed the interest to investigate the *in vitro* production of active principles in *P. corylifolia* cultures. Plant secondary metabolites product in plant cell culture can be produced on a year round basis. Elicitors are now considered as signal molecules that activate the signal-transduction cascade and lead to the activation and expression of genes related to the biosynthesis of secondary metabolites (Zhao et al., 2005).

Accumulation of medicinally important compounds can be stimulated in plant cell culture by manipulating biotic and abiotic elicitors (Sivanandhan et al., 2012). Ahmed and Baig (2014) successfully used biotic elicitors to accumulate high psoralen content in suspension cultures of *P. corylifolia*. In our previous study Methyl Jasmonate and Salicylic Acid were used as elicitors to increase psoralen content in adventitious root culture of *P. corylifolia* (Siva et al., 2015a). Different elicitors like yeast extract, myo-inositol, casein hydrolyzate, sucrose and amino acids, i.e. proline, glycine and glutamine have been used to increase psoralen content in callus cultures of *P. corylifolia* (Parast et al., 2011). Abiotic elicitors—salicylic acid, methyl jasmonate and biotic elicitors—*Aspergillus niger* and *Rhizopus oligosporus* have been proved to enhance isoflavones production in *Glycine max* (Saini et al., 2013). Ag+ has been reported to be effective elicitor for the production of tanshinones in *Salvia miltiorrhiza* Hairy Roots (Xing et al., 2015). The effects of polysaccharide elicitors such as chitin, pectin, and dextran were tested for elicitation of phenylpropanoids and naphtodianthrones in *Hypericum perforatum* shoot cultures (Simic et al., 2014). Methyl Jasmonate has been successfully used for the enhancement of flavonoid production in cell suspension culture of *H. perforatum* (Wang et al., 2015). Biotic elicitors such as yeast extract and chitosan have been shown to induce higher production of daidzein and genistein compounds in cell cultures of *P. corylifolia* (Shinde et al., 2009). Organic elicitors such as proline, yeast extract, myo-inositol and sucrose were tried to increase psoralen content in cotyledonary callus cultures of *P. corylifolia* (Ahandani et al., 2013). biotic elicitors prepared from the fungal extract (*Aspergillus niger* and *Penicillium notatum*), yeast extract and chitosan were used to accumulate high psoralen content in cell suspension cultures of *P. corylifolia* (Ahmed and Baig, 2014).

Jasmonic acid (JA) is an endogenous signal molecule that elicits plant resistance to pathogens and herbivores and stimulate secondary metabolite pathways. Jasmonic acid (JA) has been proposed to be key signaling compounds in the process of elicitation leading to the accumulation of various secondary metabolites. The Jasmonate has been previously applied in order to overproduce triterpene saponin in many plants, ginsenosides from *Panax ginseng* (Lu et al., 2001), Saikosponin from *Bupleurum falcatum* root fragments (Aoyagi et al., 2001) and soya saponin in cultures of *Glycyrrhiza glabra* (Hayashi et al., 2003). Jasmonates have also been reported to play an important role in signal transduction processes that regulate defense genes in plants (Yoon et al., 2000). The present study highlights the evaluation of psoralen content from leaves and roots of *in vitro* regenerated plants of *P. corylifolia*, using Jasmonic Acid as an organic elicitor.

2. Materials and methods

2.1. Medium preparation for seed inoculation

Medium for seed inoculation was prepared in culture tube (Borosil, India) containing growth regulator-free Murashige and Skoog (MS) (Murashige and Skoog, 1962) and Gamborg B_5 vitamins (Gamborg et al., 1968) with 3% (w/v) sucrose (Hi-media, India) and 0.8% agar (Hi-media, India). The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or HCl before autoclaving. Approximately, 10 mL of medium was dispensed in each culture tube and autoclaved at 1.06 kg cm^{-2} at 121 °C for 15 min.

2.2. Surface sterilization and inoculation of seeds

Fresh and mature seeds of *P. corylifolia* were collected from Experimental Garden, Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The seeds were subjected to heat treatment as described in our previous work (Siva et al., 2014). Seeds were washed thoroughly in tap water for 10 min, followed by soaking in soap solution (2% Teepol – commercial soap solution) for 5 min and then seeds were kept under running tap water for 30 min. The seeds were then disinfected with 70% ethanol for 45 s and rinsed with double distilled water for 3 times, followed by 0.1% (w/v) aqueous mercuric chloride treatment exposure for 5 min. The surface-disinfected seeds were inoculated into autoclaved hormone free MS medium.

Initially the cultures were maintained in dark condition for 48 h at 25 ± 2 °C and then shifted to 16 h light and 8 h dark photoperiod condition with the light intensity of 3000 lx.

2.3. Medium preparation for multiple shoot induction

Medium for multiple shoot induction was prepared in culture tube (Borosil, India) Murashige and Skoog (MS) (Murashige and Skoog, 1962) and Gamborg B_5 vitamins (Gamborg et al., 1968) with 6% (w/v) sucrose (Parast et al., 2011) and 0.8% agar (Hi-media, India). Plant growth regulators for multiple shoot induction and elongation along with Jasmonic Acid were added to the medium and pH was adjusted to 5.8 using 0.1 N NaOH or HCl before autoclaving. Approximately, 10 mL of medium was dispensed in each culture tube and autoclaved at 1.06 kg cm^{-2} at 121 °C for 15 min.

2.4. Inoculation of shoot tip explant for multiple shoot induction

Shoot tip explants were isolated from 15 days old aseptically grown seedlings and inoculated on MSB_5 medium supplemented with with 6% (w/v) sucrose (Parast et al., 2011), 0.5–3.0 mg/L TDZ

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