SUPPLEMENTARY MATERIAL

Enhanced daidzin production from jasmonic and acetyl salicylic acid elicited hairy root cultures of *Psoralea corylifolia* L. (Fabaceae)

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Abstract

Daidzin (7-O-glucoside of daidzein) has several pharmacological benefits in herbal remedy, as antioxidant and shown antidipsotropic activity. Hairy root culture of *Psoralea corylifolia* L. was developed for biomass and enhanced daidzin production using signaling compounds such as jasmonic acid (JA) and acetyl salicylic acid (ASA). Best response of 2.8-fold daidzin (5.09% DW) with 1 μ M JA treatment after second week and 7.3-fold (3.43% DW) with 10 μ M JA elicitation after tenth week was obtained from hairy roots compared to untreated control. ASA at 10 μ M promoted 1.7-fold increase in daidzin (1.49% DW) content after seventh week compared to control (0.83% DW). Addition of 25 μ M ASA resulted in 1.44% DW daidzin (1.5-fold increase) with 0.91% DW in control after fifth week and 1.44% DW daidzin (2.3-fold increase) after eighth week when compared to untreated control (0.62% DW). Reduced biomass with increased daidzin content was facilitated by elicited hairy root cultures.

Keywords: acetyl salicylic acid; daidzin; elicitation; hairy root culture; jasmonic acid; yield enhancement

Experimental

Hairy root culture and maintenance

Hairy root cultures of *Psoralea corylifolia* developed earlier in our laboratory (Abhyankar et al. 2005); were maintained on hormone free MS (Murashige and Skoog 1962) solid medium containing 0.9% (w/v) agar-agar supplemented with 3% (w/v) sucrose. Hairy root cultures, was sub-cultured at four-week interval and maintained in the dark at $25 \pm 1^\circ\text{C}$ temperature. Profuse growth of hairy roots was obtained on hormone free MS medium. Till date, hairy root cultures are maintained at culture room conditions using flasks of different sizes in large numbers.

Elicitor preparation and treatment

Elicitation was carried out using jasmonic acid (JA) and acetyl salicylic acid (ASA) at varying concentrations. Stock solutions of JA and ASA, was prepared by dissolving 250 mg of each signal compound in 500 μL of dimethyl sulfoxide (DMSO) for JA and absolute ethanol in case of ASA, respectively. Final stock solution of 25 mg/mL was prepared by adding sterile double distilled water. Both the stock solutions were filter-sterilized through 0.22 μm filter before addition into the cultivation medium. Generally, in our laboratory we are maintaining hairy roots by sub-culturing them with a culture passage of 4-weeks interval. Subsequent to subculture, 21 day old hairy roots were used for elicitation experiments. The approximate inoculum size of 0.06 g of hairy roots were cultured on the media supplemented with different concentrations of JA (1, 5, 10, 25, 50 and 100 μM) and ASA (1, 10 and 25 μM) along with untreated controls. The growth characteristics of the hairy root cultures and daidzin content were determined.

Determination of biomass

After elicitor treatment, hairy roots were harvested at seven-day interval extending up to tenth week for the analysis of biomass and daidzin content. Hairy roots were carefully separated from the solid medium, rinsed with distilled water, subsequently soaked using blotting paper and fresh weight (FW) was taken. Dry weight (DW) was estimated after roots were air dried for three weeks at room temperature ($28 \pm 2^\circ\text{C}$). Growth kinetics analysis of hairy roots was carried out from first to tenth week by taking fresh weight (FW) and dry weight (DW) of hairy root biomass.

Extraction of tissue samples and estimation of daidzin content

Daidzin (7-O-glucoside of daidzein) was extracted from the dried elicitor treated and untreated (control) hairy roots using high-performance liquid chromatography (HPLC) grade methanol as per Abhayankar et al. (2005) with certain modifications. Dried samples (25 mg) of hairy roots were powdered and extracted with 2.5 mL of methanol at room temperature for 2 days. The extract was filtered through Whatmann filter paper No. 1, and concentrated to 1 mL by evaporation. The solution obtained was further filtered through a 0.45 μm membrane (Millex HV, Millipore, Ireland) and then subjected to HPLC system for analysis. The daidzin fractions were analyzed using HPLC (Waters, USA). Separation of compounds was carried out by reverse phase C_{18} column (250 mm x 4.6 mm, 5 μm) eluting methanol as mobile phase at 1 mL min^{-1} flow rate with 20- μL injection volume of sample. The column was maintained at 25°C . The software used was Empower pro. Daidzin was detected with a photodiode array (PDA) detector at a wavelength of 254 nm with retention time of 2.6 ± 0.3 min. The solvents were degassed before use and commercially available daidzin (Sigma, USA) was used as an authentic standard. Daidzin content was expressed as %DW of the sample under specific treatment and study. Statistical analysis of data using mean, standard deviation and standard error was calculated using SigmaPlot software.

References

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Figure S1-S6

Figure S1 Effect of jasmonic acid (JA) on *P. corylifolia* hairy root growth (FW in gm). Bars represent the mean $\pm$ SE

Figure S2 Effect of jasmonic acid (JA) on *P. corylifolia* hairy root growth (DW in gm). Bars represent the mean $\pm$ SE

Figure S3 Effect of jasmonic acid (JA) on daidzin production (%DW) in hairy root cultures of *P. corylifolia*. Bars represent the mean $\pm$ SE

Figure S4 Effect of acetylsalicylic acid (ASA) on *P. corylifolia* hairy root growth (FW in gm). Bars represent the mean $\pm$ SE

Figure S5 Effect of acetylsalicylic acid (ASA) on *P. corylifolia* hairy root growth (DW in gm). Bars represent the mean $\pm$ SE

Figure S6 Effect of acetylsalicylic acid (ASA) on daidzin production (%DW) in hairy root cultures of *P. corylifolia*. Bars represent the mean $\pm$ SE