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Correlation of different spectral lights with biomass accumulation and production of antioxidant secondary metabolites in callus cultures of medicinally important *Prunella vulgaris* L.

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ABSTRACT

Light is one of the key elicitors that directly fluctuates plant developmental processes and biosynthesis of secondary metabolites. In this study, the effects of various spectral lights on biomass accumulation and production of antioxidant secondary metabolites in callus cultures of *Prunella vulgaris* were investigated. Among different spectral lights, green light induced the maximum callogenic response (95%). Enhanced fresh biomass accumulation was observed in log phases on day-35, when callus cultures were exposed to yellow and violet lights. Yellow light induced maximum biomass accumulation (3.67 g/100 ml) from leaf explants as compared to control (1.27 g/100 ml). In contrast, violet lights enhanced biomass accumulation (3.49 g/100 ml) from petiole explant. Maximum total phenolics content (TPC; 23.9 mg/g-DW) and total flavonoids content (TFC; 1.65 mg/g-DW) were observed when cultures were grown under blue lights. In contrast, green and yellow lights enhanced total phenolics production (TPP; 112.52 g/100 ml) and total flavonoids production (TFP; 9.64 g/100 ml) as compared to control. The calli grown under green, red and blue lights enhanced DPPH-free radical scavenging activity (DFRSA; 91.3%, 93.1% and 93%) than control (56.44%) respectively. The DFRSA was correlated either with TPC and TFC or TPP and TFP. Furthermore, yellow lights enhanced superoxide dismutase (SOD), peroxidase (POD) and protease activities, however, the content of total protein (CTP) was higher in control cultures (186 μg BSAE/mg FW) as compared to spectral lights. These results suggest that the exposure of callus cultures to various spectral lights have shown a key role in biomass accumulation and production of antioxidant secondary metabolites.

1. Introduction

Plants are exposed to a variety of environmental factors including low and high temperatures, light intensity, alkalinity, acidity, drought and oxidative damages. These changes induce stresses in plants and trigger a series of morphological, physiological, biochemical and molecular variation in plants [1]. A number of factors like temperature; light, pH, aeration and agitation affecting the production of metabolites have been studied extensively [2–4]. By optimizing the cultural conditions, several products accumulated at elevated levels in cultured cells than in intact plants [5–6]. Manipulations of nutritional components as well as physical factors received by the culture are the primary approaches for optimizing the culture productivity.

Light is among the most important physical factors and its quality plays a fundamental role in photosynthesis and morphogenesis that alters plant architectural development [7]. It also plays a significant role in regulating the synthesis of valuable phytochemicals [8–10]. In many elite plant species, application of periodic light to *in vitro* cultures modified their regeneration potential and enhanced the production of desirable metabolites. Light has a direct relationship with chlorophyll content and its fluctuation sometimes enhances secondary metabolites production [11]. Light intensity and selective wavelength stimulated the production of secondary metabolites in many medicinal plants [12–13]. Fluorescent lamps are the major source of light in plant cell, tissue and organ culture [14]. Various reports confirmed the morphological and physiological effects of quality of light and these responses vary considerably depending upon plant species.

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Prunella vulgaris (*P. vulgaris*) is important medicinal plant in the family Lamiaceae and commonly known as self-heal due to its faster effects on wound healing [15]. Traditionally, it is used for many ailments in various systems of medicines [16–17]. In Unani formulations, the floral parts of Prunella are widely exploited for curing cold, headache and sore throat infections [18]. It is commonly used in Korean traditional medicine to treat goiter, nephritis and oedema. In Chinese medicine it is used for the treatment of thyroid gland malfunction and also used as detumescence, antifebrile, sedative and mastitis [19]. Various modern clinical studies demonstrated that Prunella has a wide spectrum of biological effects including the production of cytokines and T lymphocytes, anti-viral, anti-tumor and anti-inflammatory activities [16,20–22]. The medicinal importance of this species can be judged from its anti-spasmodic, anti-cancerous, anti-septic, anti-rheumatic, anti-HIV and anti-HSV properties [19,18]. The increasing demand of the medicinal plants including *P. vulgaris* for remedial usage has led to the increased burden on plant's collection from their natural repositories. Unfortunately, lesser information is available about the production of *P. vulgaris* both in field and *in vitro* conditions.

Therefore, the aim of the present study was to develop callus culture under the influence of various spectral lights because callus cultures minimized the time required for the regeneration of whole plantlets and production of secondary metabolites which are either produced in limited quantities in parental plants or difficult to synthesize under laboratory conditions. This is the first successful report on callus development and production of secondary metabolites under the influence of various spectral lights. Furthermore, various spectral lights were applied to test total phenolics content, total phenolics production, total flavonoids content, total flavonoids production, antioxidant activity, superoxide dismutase and peroxidase activities and content of total protein in callus cultures of *P. vulgaris*.

2. Material and Methods

2.1. Development of Callus Cultures

To develop callus cultures, leaf explants were obtained from *in vitro* seed derived plantlets of *P. vulgaris*. The age of the donor plant was 30 days and approximately, 3–4 mm^2 leaf portions were cut out and subsequently cultured on Murashige and Skoog medium [23] supplemented with 2.0 mg l^{-1} of NAA. 30 g l^{-1} sucrose was added to MS media as a carbon source. The pH of the media was adjusted to 5.7 through a pH meter (Eutech Instruments pH 510, Singapore). Thereafter, 8 g l^{-1} agar (Agar Technical LP0013, Oxoid, Hampshire, England) was added to the media and sterilized at 121 °C for 20 min by using autoclave (Systec VX 100, Germany). All cultures were maintained in a growth room at temperature of 25 ± 2 °C under a 16/8 h photoperiod with a light intensity ranging from ~40–50 μmol m^{-2} s^{-1} provided by fluorescent tube lights (20 W, Toshiba FL20T9D/19; 380–780 nm). MS medium with 2.0 mg l^{-1} of NAA was also used as control.

2.2. Spectral Lights Treatments

To monitor the effect of various spectral lights on callus induction, the method of Tariq et al. [24] was followed. Different illumination sources used were sole; blue tubes (220 V; 50 Hz, Keliang Ltd.) 380–560 nm, green tubes (40 W Litex) 480–670 nm, yellow tubes (36 W, Philips Ltd.) 530–780 nm at an intensity ranging from approximately 40–50 mol m^{-2} s^{-1} or 10,240 lx and red fluorescent tubes (25 W, Binxiang) 610–715 nm or 640 lx. Light intensity was measured by using a Lux meter (SU10, Jeiotech) under the light source. MS medium containing 2.0 mg l^{-1} of NAA, placed under the white fluorescent tube lights (photoperiod 16/8 h) was used as control. After 3 weeks of culture, the number of responding explants was recorded. Callus subculturing was performed on media with same PGR composition.

2.3. Establishment of Growth Curve for Biomass Accumulation

Data regarding growth kinetics was collected with 7 days interval for a period of 49 days. Growth curve was established for the accumulated biomass of the rapidly growing calli from leaf and petiole explants in response to different spectral lights. To determine fresh weight (FW), calli were carefully removed from cultured flasks, slightly washed with sterile distilled water, pressed gently on filter paper (Whatman Ltd., England) to remove excess water and finally weighed (Sortorious digital balance; Germany). Similarly, for dry weight (DW) determination, calli were dried in an oven (Thermo Scientific; Germany) at 50 °C and finally weighed. Fresh and dry weights of calli were expressed in g/100 ml.

2.4. Determination of Total Phenolics and Flavonoids Content

The dried calli obtained from different treatments were grounded in fruit juicer for extract preparation. Ethanol was used as an extraction solvent. 10 mg of dried powder was mixed with 10 ml of ethanol and kept for 1 week with periodic shaking. These solutions were centrifuged (14,000 rpm) for 15 min and the supernatant was used for the determination of TPC and TFC. TPC in each sample was determined by using the recent methods of Ahmad et al. [11]. Briefly, 0.1 ml (2 N) Folin–Ciocalteus reagent was mixed with 0.03 ml extract and 2.55 ml sterile distilled water. Before incubation for 30 min, the mixture was centrifuged (10,000 rpm; 14 min) and then filtered through 45 μm membrane in a UV–visible spectrophotometer (Shimadzu-1650; Japan) cuvette. The absorbance of resulted mixture was measured at 760 nm. Gallic acid (Sigma; 1.0–10 mg/ml; $R^2 = 0.9878$) was used for plotting standard calibration curve. Results as Gallic acid equivalent (GAE) mg/g of dry weight (DW) were obtained from % TPC by using the following equation.

$$\%\text{Total phenolics content} = 100 \times (A_S - A_B)/(C_F \times D_F)$$

where A_S is the absorbance of the sample and A_B is absorbance of blank. C_F is the conversion factor from standard curve and D_F is the dilution factor.

The TFC in dried samples was determined by using the method of Ahmad et al. [11]. Ethanolic extract (0.25 ml) of the treated samples was mixed with sterile distilled water (1.25 ml) and 0.075 ml $AlCl_3$ (5%; *w*/*v*). Before incubation (5 min) and centrifugation (10,000 rpm; 14 min), the solution was mixed with 0.5 ml of NaOH (1 M). The absorbance was checked at 510 nm with a UV–visible spectrophotometer (Shimadzu-1650PC, Japan). Rutin (Sigma; 1.0–10 mg/ml; $R^2 = 0.9866$) was used for plotting standard calibration curve. The total flavonoids content was expressed as rutin equivalent (RE) mg/g-DW of extracts.

2.5. Determination of Antioxidative Enzyme Activities

To quantify stress antioxidant enzymes, fresh and vigorous calli were collected from cultured flasks under controlled conditions and quickly extracted according to the protocol of Nayyar and Gupta [25]. For fresh extract preparation, 1 g calli was taken from each treatment in a test tube and thoroughly mixed with 10 ml of extraction buffer (50 mm KH_2PO_4 buffer with 1% PVPP at pH 7). The homogenized mixture was then centrifuged (14,000 rpm) at 4 °C for 30 min. The supernatant was collected in sterile tube and later on applied for enzyme assays. Superoxide dismutase activity was investigated by using the method of Ahmad et al. [11]. The peroxidase activity was determined according to the protocol of Lagrimini [26].

2.6. Determination of Protein Content

For extract preparation, the protocol of Giri et al. [27] was followed. The antioxidant stress enzymes and protein content were determined