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Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor

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Abstract

Eleutherococcus senticosus somatic embryos were cultured in Murashiage and Skoog medium in balloon type bubble bioreactor (working volume 2-l) to investigate the effect of light on biomass, secondary metabolites, stress levels (lipid peroxidation and hydrogen peroxide (H_2O_2) and changes of antioxidant enzymes. Among the different light sources, biomass accumulation was found optimum under fluorescent (Fl) and mixed blue plus far-red (Bl + Fr) irradiation. Accumulation of eleutheroside E (51%) and E_1 (21%) was highest at red light while fluorescent light produced highest amount of total phenolic (2.7%), total flavonoid (34%) and chlorogenic acid (14%) contents compared to dark (control) grown mature embryos. Higher H_2O_2 content, malondialdehyde (MDA) content and lipoxygenase (LOX) activities was observed at red light treated embryos compared to dark (control) grown embryos. Antioxidant enzymes, monodehydroascorbate reductase (MDHAR), catalase (CAT), glutathione *S* transferase (GST) and superoxide dismutase (SOD) which are playing important role for the detoxification of harmful substances were also induced in red light irradiated embryos. However, ascorbate peroxidase (APX) took a little part in detoxification of H_2O_2 due to its sensitivity to red light. Therefore, reduced APX activity in red light treated embryos was observed. While APX activity was maximum in fluorescent light treated embryos compared to other light sources. This reflects the sensitivity of the enzyme to the different light. The study concludes that embryo could grow under different light irradiation and protect themselves from toxicity of light sources by altering various phenolic compounds and antioxidant enzymes.

Keywords: Antioxidant enzymes; Bioreactor; Eleutherosides; HPLC; Light emitting diodes; Somatic embryos

1. Introduction

Eleutherococcus is an endemic Asian genus with about 35 species distributed mainly in northeastern Asia including China, Korea, and Japan, with some members also extending to southwestern Asia [1]. Propagation of *Eleutherococcus senticosus* through conventional methods is difficult due to long period of cold, warm stratification and slow germination rate [2]. Plant tissue culture is an easy and convenient process for efficient, potential bulk propagation of whole plants. Regeneration of Siberian ginseng plants through direct somatic embryogenesis [3] and indirect embryogenic callus and cell suspension cultures has been reported [4,5]. Cell cultures of plants have long been considered to be possible sources of medicinally important secondary metabolites.

Light is an important factor affecting growth, organogenesis and the formation of plant products including both primary and secondary metabolites. The stimulatory effect of light on the formation of compounds, including flavonoid and anthocyanins has been shown in plants [6,7]. In addition to the above-mentioned effects of light on the production of plant secondary metabolites, light is also involved in regulating the secretion mechanism of secondary products [8]. It is widely believed that the synthesis of secondary metabolites in plants is part of the defense responses of plants to stress. Oxidative stress has been associated with damage of stressed plants [9]. As a result of stress, there is an immediate formation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide

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anions (O_2^-) and hydroxyl free radicals ($^{\bullet}OH$) that trigger the plant signal systems. When in excess, ROS can result in oxidation of proteins, unsaturated fatty acids and DNA, causing cellular damage and eventually cell death [9]. Plants have evolved efficient antioxidant systems to scavenge ROS. Among these systems, antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase(G-POD) and the enzymes ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR) in the Halliwell-Asada cycle are the most efficient protective mechanisms against oxidative stress [9]. In addition, a number of earlier investigation have also suggested that oxidative stress plays an important role for the synthesis of secondary metabolites in plant culture systems [10]. Different substances have been used for the production of secondary metabolites in different plants [11]. It has been also suggested that the elicitor-induced secondary metabolite synthesis of plants requires endogenous signal components such as jasmonic acid (JA) and reactive oxygen species (ROS) [12,13]. However, the role of light in the biosynthesis of secondary metabolites in plant cells is not well established. The present study described the effect of different light sources generated from light emitting diodes (LEDs) including fluorescence light on the growth of somatic embryos, production of secondary metabolites and changes of different antioxidant enzymes in bioreactor.

2. Materials and methods

2.1. Somatic embryogenesis from leaf explants and culture conditions

In vitro seedlings of *E. senticosus* were maintained in Murashiage and Skoog (MS) medium [14] without plant growth regulators (PGRs). Young leaves (2 cm in length) were collected from sub-cultured plants and cut into 5 mm × 5 mm pieces, then transferred to Petridish for embryogenic callus induction. Explants were placed on semisolid MS basal medium supplemented with 1 mg l^{-1} 2,4-D, with 3% sucrose and 0.2% gel rite for callusing and somatic embryogenesis. The medium was adjusted to pH 5.8 prior to addition of gel rite and sterilized at 121 °C for 15 min and distributed in 15 mm × 140 mm Petridishes (15 ml of medium). Cultures were maintained in dark at 25 °C and evaluated for somatic embryogenesis after 12 weeks. For proliferation of selected embryogenic cultures on solidified medium, the embryogenic calli were cultured on the same medium as described above and sub cultured at every 15 days interval.

2.2. Embryogenic cell suspension culture

Embryogenic cells of *E. senticosus* were transferred to MS liquid medium supplemented with 1 mg l^{-1} 2,4-D. The suspension cultures were sub-cultured at every 15 days interval. To induce somatic embryos, 2-week old embryogenic cell clumps were filtered through a 212 μm stainless steel sieve to remove the larger clumps. The suspension was allowed to settle for 5 min, for easier removal of the used medium. About 500 mg of cell clumps was transferred to 100 ml MS liquid medium without 2,4-D in 300 ml Erlenmeyer flasks. The cultures were incubated at 100 rpm on a gyratory shaker. The culture room temperature was maintained at 25 °C in dark.

2.3. Bioreactor culture

A balloon type bubble bioreactor (BTBB) was used for the entire somatic embryo maturation phase using embryogenic suspensions. Three liter BTB bioreactor along with 2 l of hormone free MS medium was used as a working volume. The pH of the medium was adjusted to 5.8 before autoclaving. The volume of input air was adjusted to 0.1 vvm (air volume/culture volume, min). All the bioreactors were maintained at 25 °C. Complete embryo maturation was achieved after 45 days in the same medium.

2.4. Growth of somatic embryos in bioreactors: effect of light qualities

Ten grams of globular somatic embryos were transferred to 3-l balloon type bubble bioreactor with 2-l hormone free MS liquid medium. The cultures were established and grown under different light sources of photon flux density (PFD) of 50 μmol m^{-2} s^{-1}. There were five radiation treatments: dark (C, as control), fluorescent (Fl), monochromatic red (peak emission 660 nm), monochromatic blue (Bl, peak emission of 470 nm), blue plus far-red (Bl + Fr, 1:1) light emitting diodes (LEDs). The spectral distribution of relative energy of Bl, red and Bl + Fr was determined using a quantum sensor (LI-1899, LI-COR, Lincoln, USA). Bioreactors were kept in controlled temperature (25 ± 2 °C) with 16/8 light periods in incubation chamber (Jeico Tech Bl 1000M). Three of 3-l BTB bioreactors were used under each light condition, and the collected data shown represent average values with standard error. The fresh and dry weights of mature embryos were recorded after 45 days of culture. Dry weight was determined after drying for 24 h at 60 °C. The growth ratio (GR) was determined as: GR = {harvested dry weight (g) − inoculated dry weight (g)}/inoculated dry weight (g).

2.5. Determination of eleutherosides and chlorogenic acid

Extraction and analysis of eleutherosides and chlorogenic acid were done using method of Shohael et al. [5]. These compounds were quantified by HPLC (Waters 2690 separation modules, Waters, USA) equipped with a symmetry^R C 18 (4.6 mm × 250 mm) column (Waters, USA) and using a photodiode array detector (Waters 996 photodiode array detector, Waters, USA) according to Patrick et al. [15]. Eleutherosides were separated using a flow rate of 0.8 ml/min with water and acetonitrile as the mobile phase with a linear gradient of 10% acetonitrile for 5 min, 20% acetonitrile for further 20 min, 40% for another 15 min and finally re-equilibration with 5% acetonitrile for 5 min. Quantitation was based on ultraviolet absorption at 216 nm. The peak areas corresponding to eleutherosides from the samples, with the same retention time as authentic eleutherosides B, E, E_1 and chlorogenic acid (Chromadex, USA) were integrated by comparison with an external standard calibration curve.

2.6. Determination of total phenolic and total flavonoid contents

The content of total phenolic in plant methanolic extracts were analyzed following the modification of Folin–Ciocalteu [16]. Hundred microliter of methanolic extracts were mixed with 2.5 ml deionized water, followed by addition of 0.1 ml (2N) Folin–Ciocalteu reagent. They were mixed well and allowed to stand for 6 min before 0.5 ml of a 20% sodium carbonate solution was added. The color developed after 30 min at room temperature and the absorbance was measured at 760 nm. Total flavonoid content was determined following Sakanaka et al. [17]. Briefly, 0.25 ml of the methanolic plant extract or (+)-catechin standard solution was mixed with 1.25 ml of distilled water, followed by addition of 0.75 ml of 5% sodium nitrite solution. After 6 min, 0.150 ml of 10% aluminum chloride solution was added and the mixture was allowed to stand 5 min and then 0.5 ml of 1 M sodium hydroxide was added. The mixture was brought to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm. The data were calculated using gallic acid and (+)-catechin for total phenolic and flavonoid contents, respectively.

2.7. Antioxidant enzyme assay

For determination of antioxidant enzymes activities, 0.5 g of matured somatic embryos from different treatments was homogenized in 1 ml of respective extraction buffer in a pre-chilled mortar and pestle by liquid nitrogen. The homogenate was filtered through four layers of cheesecloth and centrifuged at 22,000 × *g* for 20 min at 4 °C. The supernatant was re-centrifuged at 22,000 × g for 20 min at 4 °C. Protein concentration of the enzyme extract was determined

according to Bradford [18]. For the determination of ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) activities, embryos were homogenized in 100 mM sodium phosphate buffer (pH 7.0) containing 5 mM ascorbate, 10% glycerol and 1 mM EDTA. APX (EC 1.11.1.11) activity was determined in 1 ml reaction mixture containing 50 mM K-phosphate (pH 7.0), 0.1 mM ascorbate (extinction coefficient, 2.8 mM^{-1} cm^{-1}), 0.3 mM H_2O_2. The decrease in absorbance was recorded at 290 nm for 3 min [19]. MDHAR (EC 1.6.5.4) activity was assayed following the decrease in absorbance at 340 nm due to NADH oxidation using an extinction coefficient of 6.22 mM^{-1} cm^{-1} [20]. The 1.0 ml reaction mixture consisted of 90 mM K-phosphate buffer (pH 7.0), 0.0125% Triton X-100, 0.2 mM NADH, 2.5 mM L-ascorbic acid, and required amount of enzyme extract. One unit of ascorbate oxidase (AO) is defined by the manufacturer (units as defined by Sigma Chem. Co.) as the amount that causes the oxidation of 1 μmol of ascorbate to monodehyadroascorbate per minute. DHAR (EC 1.8.5.1) activity was measured by measuring the reduction of dehydroascorbate at 265 nm for 4 min [21]. The 1.0 ml reaction mixture contained 90 mM K-phosphate buffer (pH 7.0), 1 mM EDTA, 5.0 mM glutathione (GSH), and required amount of enzyme extract. The reaction was initiated by addition of 0.2 mM dehyadroascorbate (DHA) (extinction coefficient, 14 mM^{-1} cm^{-1}). For determination of GR, CAT, G-POD and GST activities, embryos were homogenized in 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA under liquid nitrogen. Glutathione reductase (GR; EC 1.6.4.2) activity was assayed following the reduction of DTNB at 412 nm (extinction coefficient, 13.6 mM^{-1} cm^{-1}) with some modifications as described by Smith et al. [22]. The assay mixture (1 ml) contained of 100 mM K-phosphate buffer (pH 7.5), 1 mM oxidized glutathione and 0.1 mM NADPH and enzyme extract. Catalase (CAT; EC 1.11.1.6) activity was determined following the consumption of H_2O_2 (extinction coefficient, 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min [23]. Guaiacol peroxidase (G-POD; EC 1.11.1.7) activity was measured in the reaction of oxidation of guaiacol at 470 nm (ε, 26.6 mM^{-1} cm^{-1}) according to Pütter [24]. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 0.9 mM H_2O_2, enzyme extract and 3.4 mM guaiacol as substrate. Glutathione *S* transferase (GST; EC 2.5.1.18) activity was determined by measuring the increase in absorbance at 340 nm (extinction coefficient, 9.6 mM^{-1} cm^{-1}), incubating reduced glutathione (GSH) and 1-chloro-2,4-dinithrobenzene (CDNB) as substrate, according to Drotar et al. [25]. The 1 ml reaction mixture contained 100 mM K-phosphate buffer, pH 6.25 and 0.8 mM 1-chloro-2,4-dinitrobenzene (CDNB). Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) according to the method of Beyer and Fridovich [26]. Embryos were homogenized in 1 ml cold 100 mM K-phosphate buffer (pH 7.8) containing 0.1 mM ethylenediamine tetraacetic acid (EDTA), 1% (w/v) polyvinylpyrrolidone (PVP) and 0.5% (v/v) Triton X-100. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

2.8. Measurement of H_2O_2, lipid peroxidation and lipoxygenase (LOX) activity

Hydrogen peroxide content of the embryos was measured spectrophotometrically after reaction with potassium iodide (KI) [27]. The reaction mixture consisted of 0.5 ml of 0.1% trichloroacetic acid (TCA), embryo extract supernatant, 0.5 ml of 100 mM K-phosphate buffer and 2 ml reagent (1 M KI, w/v in fresh double-distilled water). The blank probe consisted of 0.1% TCA in the absence of embryo extract. After 1 h of reaction in darkness, the absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2. Lipid peroxidation of embryos was estimated by the level of malondialdehyde (MDA) production using thiobarbituric acid (TBA) method as described by Heath and Packer [28]. One gram of embryos was homogenized with a mortar and pestle in 1 ml of 0.5% trichloracetic acid (TCA). The homogenate was centrifuged at 19,000 × *g* for 20 min. The 0.5 ml of supernatant was mixed with 2.5 ml of 20% TCA containing 0.5% TBA and heated in a boiling water bath for 30 min and allowed to cool in an ice bath quickly. The supernatant was centrifuged at 10,000 × *g* for 10 min, and resulting supernatant was used for determination of MDA content. Absorbance at 532 nm was recorded and corrected for non specific absorbance at 600 nm (ε, 155 mM^{-1} cm^{-1}). LOX activity was determined according to Axerold et al. [29]. The 1.0 ml reaction mixture contained 50 mM Tris–HCl buffer (pH 6.5), 0.4 mM linoleic acid and protein extract. The reaction was initiated by addition of required amount of extract and absorbance was recorded at 234 nm for 5 min (ε, 25 mM^{-1} cm^{-1}).

2.9. Statistical analysis

Each of the treatments was tested three times. Statistical analyses were performed according to SAS system (Version 6.21, SAS Institute Inc., Cary, NC). Statistical significance between mean values was assessed using Duncan's multiple range test. A probability of $P < 0.05$ was considered significant.

3. Results and discussion

3.1. Growths of somatic embryos and accumulation of secondary metabolites as affected by light sources

The results on the effects of different light quality on the growth and secondary metabolite accumulation of matured somatic embryos are given in Tables 1–3, respectively. Growth of embryos was significantly affected by different light treatment. The fresh weight, dry weight and growth ratio of the embryos decreased significantly under red light resulting in a embryo growth ratio of 15.2 where as fluorescent and blue plus far-red showed the highest growth ratio (19.8 for fluorescent and 19.1 for blue plus far-red) compared with dark (19.0) and blue light source (17.7). The highest fresh and dry weight was obtained under fluorescent light treated embryos (Table 1).

Recent works show that stress induced during in vitro culture changes primary metabolism leading to formation of different secondary metabolites such as flavonoids, phenolics, alkaloids, etc. [30,31]. There is also evidence that accumulation of

Table 1
Amount of total matured somatic embryos of *E. senticosus* developed in 3-l bioreactor with hormone free 2-l MS medium as affected by different light quality

Light quality	Biomass ($g\ l^{-1}$)			Growth ratio[a]
	Fresh weight	Dry weight	% Dry weight	
Dark	105.6 a ± 1.1	10.6 ab ± 0.82	10.06 a ± 0.74	19.05 a ± 1.84
Fluorescent	109.0 a ± 0.8	11.1 a ± 1.62	10.13 a ± 1.12	19.81 a ± 1.49
Blue	104.4 a ± 1.2	9.9 ab ± 0.95	9.52 a ± 0.98	17.77 ab ± 1.51
Red	93.7 b ± 0.8	8.6 b ± 0.58	9.18 a ± 0.88	15.22 b ± 1.55
Blue + far-red (1:1)	108.0 a ± 1.3	10.7 ab ± 0.63	9.78 a ± 0.84	19.11 a ± 2.14

Data were taken after 45 days of culture. Values represent mean ± S.E. ($n = 3$). Different letters in each column differ significantly according to DMRT test ($P \leq 0.05$).

[a] Growth ratio is the quotient of the [{harvested dry weight (g) − inoculated dry weight (g)}/inoculated dry weight (g) of the inoculums].

Table 2
Content of total phenolic, total flavonoid and chlorogenic acid on matured somatic embryos of *E. senticosus* affected by different light qualities in bioreactor

Light quality	Contents (mg g^{-1} dry weight)		
	Total phenolic	Total flavonoid	Chlorogenic acid
Dark	16.91 a ± 0.77	10.56 b ± 0.33	1.04 b ± 0.02
Fluorescent	17.38 a ± 0.43	15.87 a ± 0.69	1.21 a ± 0.20
Blue	10.89 b ± 0.47	6.55 c ± 0.37	0.63 d ± 0.19
Red	15.98 a ± 0.08	10.50 b ± 0.38	0.99 b ± 0.11
Blue + far-red (1:1)	11.37 b ± 0.32	6.67 c ± 0.31	0.80 c ± 0.09

Data were taken after 45 days of culture. Values represent mean ± S.E. ($n = 3$). Different letters in each column differ significantly according to DMRT test ($P \leq 0.05$).

phenolic compounds is related to the oxidative stress [32–34]. Inductions in the levels of phenolic compounds are of greater importance in the prevention of stress-induced oxidative damage as they are playing an important role to the removal of toxic substances [35,36]. Light source, which can create stresses on the embryo, may act as an elicitor of plant defense response so as to stimulate the secondary metabolite synthesis. This hypothesis is supported by the production of hydrogen peroxide produced by the treated embryos (Fig. 1C). The production of H_2O_2 was less in dark grown embryos (without any light), whereas the light treated embryos showed higher metabolic activities. The production of ROS such as H_2O_2 in plants, known as the oxidative burst, is an early event of plant defense response to different stress and also acts as a secondary messenger to signal subsequent defense reactions in plants [32]. In our study, total phenolic, total flavonoid and chlorogenic acid accumulation were significantly higher in fluorescent light 17.38, 15.87, 1.21 mg g^{-1} DW, respectively (Table 2). On the other hand, blue light inhibited the accumulation of total phenolic, flavonoid and chlorogenic acid compared with red, fluorescent and blue plus far-red light treatment. The major components of eleutherosides were B, E and E_1 were quantified by HPLC (Table 3). In our results, blue light enhanced the maximum accumulation of eleutheroside B (27.9 μg g^{-1} DW), however red light produced the highest amount of eleutheroside E (54.5 μg g^{-1} DW) and E_1 (50.4 μg g^{-1} DW) as compared to other light sources and control (dark), which are known to be important molecules of pharmacological significance. The

Table 3
Effects of light qualities on eleutherosides production of matured somatic embryos of *E. senticosus* in bioreactor

Light quality	Eleutherosides (μg g^{-1} dry weight)			
	B	E	E_1	Total
Dark	21.3 b ± 1.1	26.7 c ± 2.1	39.7 b ± 1.7	87.6 d ± 1.5
Fluorescent	23.1 a ± 1.5	42.9 b ± 2.5	48.6 a ± 2.1	114.6 b ± 2.1
Blue	27.9 a ± 1.5	25.0 c ± 1.9	24.6 c ± 1.1	77.4 e ± 1.6
Red	14.9 c ± 0.9	54.5 a ± 2.6	50.4 a ± 1.5	119.7 a ± 2.9
Blue + far-red (1:1)	22.6 b ± 1.3	37.2 b ± 1.8	35.8 b ± 1.7	95.5 c ± 2.5

Data were taken after 45 days of culture. Values represent mean ± S.E. ($n = 3$). Different letters in each column differ significantly according to DMRT test ($P \leq 0.05$).

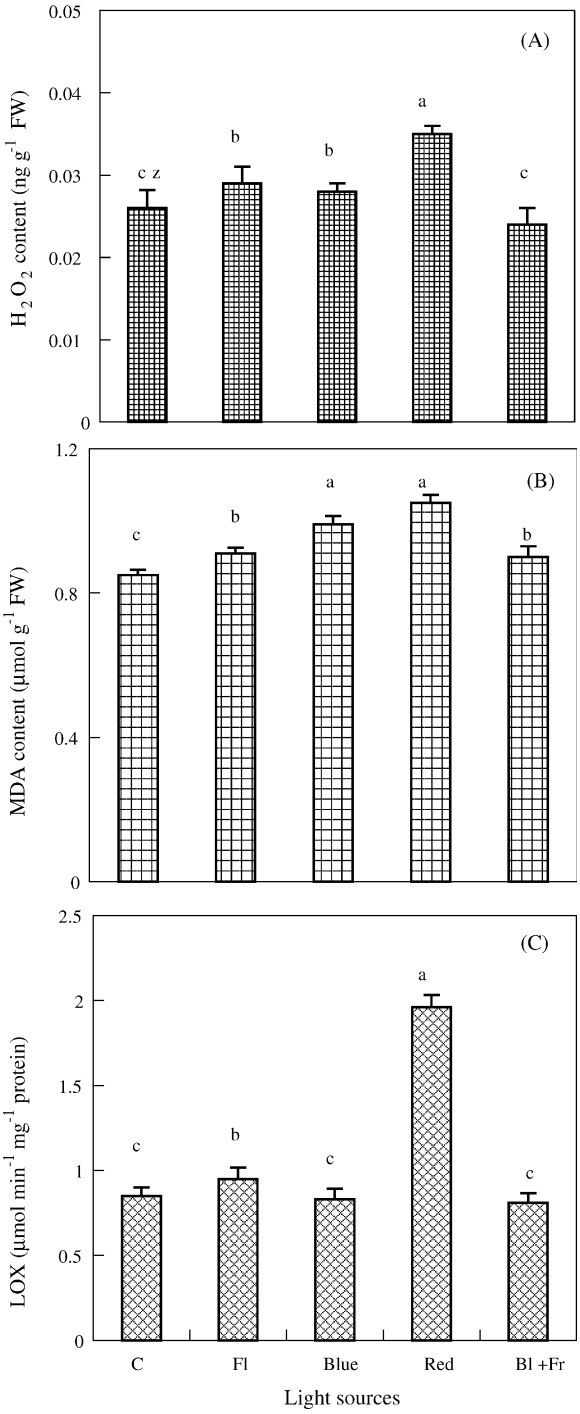

Fig. 1. H_2O_2 content (A), MDA content (B) and LOX activity (C) in matured somatic embryos cultured in bioreactor affected by different light quality (cont: dark, Fl: fluorescent, Bl: blue, Bl + Fr: blue + far-red). [z]Different letters in each bar differ significantly according to DMRT test ($P \leq 0.05$).

variation of induction of secondary metabolites under different light sources may be due to the different action of light during growth of somatic embryos. Since biomass accumulation was affected significantly by red light and accumulates the maximum amount of eleutherosides (E and E_1) might be due to H_2O_2 accumulation (Fig. 1A). The formation of H_2O_2 is the most important phenomenon of signal transudations induced by

stress conditions, which changes the content of redox agents and the redox state of cells [37]. Our observation in the increase in H_2O_2 content with a corresponding increase in the secondary metabolites under red light is in accord with earlier reports [38] in suspension cultures of *Taxus chinensis* var. *mairei* induced by oligosaccharide from *Fusarium oxysprum*. Similar to the present results, stimulatory effects of light on the formation of secondary metabolites have been reported in *Perilla frutiescnens* [7] and *Artimisia annua* [39]. Therefore, the accumulation of ROS signals in embryos treated by red light induced the synthesis of secondary metabolite.

Formation of malondialdehyde (MDA) (Fig. 1B) was considered as a measure of lipid peroxidation that was induced by red light treated embryos. MDA, a decomposition product of polyunsaturated fatty acids hydroperoxides, has been utilized very often as a suitable biomarker for oxidative stress. This hypothesis is also confirmed by the higher LOX activity in red light treated embryos (Fig. 1C). Total MDA and LOX activity in red light treated embryos were significantly higher than control indicating higher stress levels. These results indicate that lipid peroxidation occurred in *E. senticosus* embryos, probably as a consequence of higher amount of H_2O_2 levels (Fig. 1A). H_2O_2 may function as a signal for the induction of defence systems and could enhance secondary metabolite production [40]. Thus it is speculated that a cascade of events including lipid peroxidation, accumulation of H_2O_2 content may be involved in the initiation of secondary metabolite production. In view of this it appears that oxidative stress is a pre-requisite for secondary metabolite synthesis.

Table 4 shows the results of responses of antioxidant enzyme activities of *E. senticosus* embryos. APX activities showed higher value in fluorescent and blue light irradiated embryos compared to red and blue plus far-red irradiated embryos. The MDHAR activities of all the treated embryos showed higher value than the red light treated embryos. On the other hand, DHAR showed higher activities in fluorescent treated embryos than the dark grown embryos. GR activities, on the other hand, showed a much milder effect in dark grown embryos and blue, red and blue plus far-red treated cultures increased 46%, 36% and 48%, respectively compared to dark grown embryos. CAT activities showed higher value in red light treated embryos followed by blue light while other light sources remained unchanged. G-POD activities showed higher value in all cases except in blue plus far-red irradiated embryos where G-POD decreased significantly. The average SOD activity was 0.39 units in dark treated embryos. Among the irradiation, red light induced the highest SOD activity with 0.55 units, followed by fluorescent light with 0.48 units. On the other hand blue plus far-red treated embryos showed the lowest activity with 0.34 units.

The different effects exhibited by different light sources are indicative of different level of activities and secondary metabolite induction. Superoxide dismutase first catalyzes the conversion of O_2^- into H_2O_2 and O_2. The increasing SOD activity in red light treated embryos might be a defensive response, which could reflect a lower O_2^- production or a higher capacity for elimination of O_2^-. The red light irradiated embryos showed coordinated increase in the activities of SOD (a H_2O_2-generating enzyme) and G-POD and CAT (H_2O_2-scavenging enzymes). Ascorbate peroxidase (APX) is a member of ascorbate–glutathione cycle, and plays a crucial role in eliminating poisonous H_2O_2 from plant cells. In this study, red light treated embryos inhibited APX activity in. This might be due to the harmful effects of over production of H_2O_2 or its poisonous active oxygen derivatives, because of manifold increase in SOD activity of the red light treated embryos. On the other hand, MDHAR and DHAR responsible for the regeneration of reduced ascorbate, also inhibited in red light treated embryos that might be due to lower APX activity as, APX requires ascorbate as substrate [41]. The GR and GST activity was higher in red light treated embryos in comparison to those of other treatments. This might suggest a higher rate of production of ROS in the red light treated somatic embryos. Increased GR activity in plant has been reported to be closely related with tolerance capacity of the plants by maintaining the GSH level [42]. Other enzymes such as CAT and G-POD are also involved in removing of H_2O_2. The stimulated activities of these enzymes (G-POD and CAT) and reduced APX activity found in this study led to the conclusion that elimination of H_2O_2 in embryos was achieved by G-POD and CAT. However, CAT activity was influenced more than G-POD in red light indicates that CAT was more involved to protect the embryos from the stress conditions. In addition, G-POD also plays an important role in the coupling of indole alkaloids synthesis under different stresses in different plants [43]. Higher G-POD activity, concomitant with the increased accumulation of eleutheroside E and E_1, were found in somatic embryos of *E. senticosus* as compared to other treatments. Hence, there was a close relationship between the G-POD activity and eleutheroside accumulation.

Table 4
Activities of different antioxidant enzymes in matured somatic embryos cultured in bioreactor affected by different light quality

Light quality	Enzyme activities (μmol min^{-1} mg^{-1} protein)							Unit (mg^{-1} protein)
	APX	MDHAR	DHAR	GR	CAT	G-POD	GST	SOD
Dark	12.14 a ± 0.66	30.78 c ± 1.0	12.25 b ± 0.82	32.13 c ± 1.8	2.33 c ± 0.21	0.66 b ± 0.04	14.4 a ± 0.57	0.39 b ± 0.08
Fluorescent	14.80 a ± 0.77	36.75 b ± 1.0	15.61 a ± 1.0	34.80 c ± 1.0	2.50 c ± 0.18	0.72 b ± 0.02	15.2 a ± 0.67	0.48 a ± 0.06
Blue	10.61 b ± 0.31	40.20 a ± 0.6	11.78 b ± 1.00	56.02 b ± 1.0	3.24 b ± 0.20	0.51 b ± 0.02	14.6 b ± 0.74	0.43 b ± 0.10
Red	8.23 b ± 0.57	28.74 c ± 1.3	8.27 c ± 0.55	50.08 b ± 1.3	4.50 a ± 0.67	0.93 a ± 0.03	18.5 a ± 0.88	0.55 a ± 0.11
Bl + Fr (1:1)	8.52 b ± 0.39	35.68 b ± 1.2	13.99 b ± 0.67	62.45 a ± 3.0	2.54 c ± 0.56	0.35 c ± 0.02	16.2 a ± 0.53	0.34 b ± 0.09

Data were taken after 45 days of culture. Values represent mean ± S.E. ($n = 3$). Different letters in each column differ significantly according to DMRT test ($P \leq 0.05$).

In conclusion, this study shows that red light induces eleutheroside E and E_1 production but with a reduction in dry weight of embryo, higher oxidative stress levels and increased antioxidant activities in *E. senticosus* embryo occurred. On the other hand, fluorescent light is useful for the production of phenolic, total flavonoid and chlorogenic acid. This study also shows that differences in APX, MDHAR, DHAR, GR, CAT, G-POD, GST and SOD activities in somatic embryos of *E. senticosus* plants could be attributed to differences in mechanisms underlying oxidation stress injury and subsequent tolerance to different light sources. The induction or suppression of antioxidant activities provides evidence for occurrence of oxidative burst in different light treated embryos and variation of secondary metabolite accumulation in somatic embryos of *E. senticosus*.

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