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Research Report

Blue LED Light Enhances Growth, Phytochemical Contents, and Antioxidant Enzyme Activities of *Rehmannia glutinosa* Cultured In Vitro

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Abstract. The objective of the current study is to determine the effect of light quality on enhancement of growth, phytochemicals, antioxidant potential, and antioxidant enzyme activities at in vitro cultures of *Rehmannia glutinosa* Libosch. In vitro-grown shoot tip explants were cultured on the plant growth regulator (PGR)-free Murashige and Skoog (MS) medium and cultured under a conventional cool white fluorescent light (control), blue light emitting diode (LED) light or red LED light. After four weeks, the growth traits along with total phenol content, total flavonoid content, free radical scavenging activities, and antioxidant enzyme activities were measured. Interestingly, the blue or red LED treatments showed a significant increase in growth parameters compared with the cool white florescent light. In addition, the LED treatments increased the total phenol and flavonoid levels in leaf and root extracts. Furthermore, data on the total antioxidant capacity, reducing power potential, and DPPH radical scavenging capacity also revealed the enhancement of antioxidant capacity under both blue and red LED treatments. Especially, the blue LED treatment significantly increased the antioxidant enzyme activities in both the leaf and root, followed by the red LED treatment. Modulation in the spectral quality particularly by the blue LED induced the antioxidant defense line and was directly correlated with the enhancement of phytochemicals. Therefore, the incorporation of blue or red LED light sources during in vitro propagation of *R. glutinosa* can be a beneficial way to increase the medicinal values of the plant.

***Additional key words*:** antioxidant potential, light emitting diodes, metabolite enhancement, secondary metabolites

Introduction

Rehmannia glutinosa Libosch. (Chinese foxglove) is a medicinally important herb, and widely distributed in the mountains of Korea, China, and Japan. The root extracts of *R. glutinosa* has been employed to treat anemia, inflammation, hypoglycemia, and hypertension since antiquity (Zhang et al., 2008). The medicinal importance of the *R. glutinosa* roots are attributed to the occurrence of secondary metabolites such as catalpol, leonuride, aucubin, melittoside, rehmaglutin, etc. with pharmaceutical importance (Chung et al., 2006; Zhang et al., 2008). Because of the comprehensive medicinal values, *R. glutinosa* has been considered as a top grade medicinal herb in traditional Chinese medicine and widely used to strengthen blood system, immune system, endocrine system, cardio-vascular system, and nervous system (Zhang et al., 2008). However, irrespective of its medicinal values, the cultivation of this plant has been limited due to seed dormancy, virus infection, low yields, and slow plant growth rate (Park et al., 2009). In order to overcome these difficulties plant tissue culture techniques can be incorporated for the mass propagation of *R. glutinosa*. In addition, most of the existing reports are limited to the root-based antioxidant analysis and also very few have examined the phenol and flavonoid contents of *R. glutinosa* (Piatczak et al., 2014). The importance of medicinal plants lies on the presence of bioactive secondary metabolites such as phenols and flavonoids that can readily act as free radical scavengers. Free radicals

are highly reactive molecules produced as the intermediates during metabolic reactions (Machlin and Bendich, 1987). The presence of excess amount of reactive free radicals in the human body causes oxidative damages leading to various lethal disorders (Kinsella et al., 1993). Furthermore, the in vitro environment offers a plausible and convenient atmosphere to understand the mechanism of secondary metabolite synthesis and accumulation without the intrusions of external environment.

Until date no reports are available on the enhancement of phytochemicals and antioxidant property of *R. glutinosa* by light quality using blue and/or red light emitting diodes (LEDs). Among several environmental factors, light influences various metabolic activities of the plant, especially under in vitro environment (Kozai et al., 1997). Thus, the synthesis and accumulation of phytochemicals with medicinal uses can be elicited using different light spectra. On comparison with the conventional cool white fluorescence light source used in plant tissue cultures, LED lights possess various advantages such as less heat radiation, energy efficiency, monochromatic spectrum, and longer life span (Kim et al., 2004; Samuoliene et al., 2012). Amongst various light spectra, blue (450 nm) and red (650 nm) wavelengths are the primary spectral wavelengths and highly influence the primary and secondary metabolism of plants (Johkan et al., 2010). Therefore, the objective of the current study was to enhance the phytochemicals and antioxidant properties of *R. glutinosa* during in vitro propagation using blue and red LEDs.

Materials and Methods

Culture Conditions and Light Treatments

In vitro-grown, four weeks old *R. glutinosa* shoot tips were inoculated on the plant growth regulator (PGR)-free Murashige and Skoog (1962) (MS) medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. The pH was adjusted to 5.70 using 0.1 N NaOH or 0.1 N HCl before autoclave at 121°C for 15 min. All the cultures were maintained at 25°C under a 16 h photoperiod with 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and 80% relative humidity (RH). Light treatments were provided from the top by either cool white fluorescent light (FL), or monochromatic spectral light emitting diodes (LEDs) such as red (450 nm) or blue (650 nm). The plants were harvested after four weeks and their growth parameters such as stem length, root length, number of roots, length of vigorously growing third leaf with ruler, number of leaves, and fresh and dry weights were measured. Similarly, phytochemicals content, enzymes activity, and antioxidant activity were measured in fresh weight basis.

Chlorophyll Content Estimation

Chlorophyll content was measured colorimetrically by following the procedures of Arnon (1949). Briefly, 100 mg leaf tissue was extracted with an 80% (v/v) acetone and the absorbance was measured at 645 and 663 nm by a UV-spectrophotometer (Uvikon 992, Kotron Instrumentals, Milano, Italy).

Plant Extracts Preparation and Phytochemical Estimation

For phytochemical and antioxidant assays, the leaf and root of in vitro-grown *R. glutinosa* were harvested after four weeks of light treatments and extracted with methanol according to Gracia-Perez et al. (2012) with slight modifications. Briefly, 0.5 g of plant tissue was homogenized using liquid nitrogen and extracted with 5 mL of 80% methanol with continuous shaking (Model 200FL, Koencon shaking incubator, Hanam City, Korea) for 5 h at 200 rpm. Then the extract was centrifuged at 1,000 g for 12 min and the supernatant was employed for the assays. The total phenol content (expressed in gallic acid equivalent) of the extract was estimated by the Folin-Ciocalteu (FC) principle according to Kumaran and Karunakaran (2007). Aliquot of the extracts (0.1 mL) made up to 1 mL with distilled water was mixed with 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (7.5%). The reaction mixture was incubated in dark for 40 min and the absorbance was recorded at 725 nm. The total phenol content was estimated using standard gallic acid calibration curve.

The total flavonoid content (expressed in quercetin equivalent) was determined by aluminum chloride method outlined by Gracia-Perez et al. (2012). Briefly, samples (0.1 mL) were made up to 1 mL with 80% methanol and used for the analysis by adding 1 mL of 2% aluminum chloride solution. The absorbance of the reaction mixture was measured at 415 nm after 30 min incubation and the total flavonoids were determined from the standard quercetin calibration curve.

Total Antioxidant Capacity Analysis

Total antioxidant activity of the extracts was analyzed using the phosphomolybdenum method according to Kumaran and Karunakaran (2007). For the reduction reaction, 0.1 mL extract was mixed with 3 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was incubated for 90 min at 95°C and the absorbance was determined at 695 nm. The phosphomolybdenum reduction (total antioxidant activity) was expressed as microgram of ascorbic acid equivalent per milligram of fresh weight ($\mu g \cdot mg^{-1} \cdot$AAE FW).

Reducing Power Capacity Assessment

The reducing power capacity was determined by the procedure of Kumaran and Karunakaran (2007) with slight

modifications. Briefly, 0.1 mL of extracts in 1 mL of phosphate buffer was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The reaction was initiated by maintaining the tubes at 50°C for 20 min in a hot waterbath. After incubation, the reaction was terminated by the addition of 2.5 mL ice cold trichloroacetic acid (10%, v/v). The supernatant (2.5 mL) was extracted after centrifugation at 1,000 rpm for 10 min and mixed with equal volume of DH_2O and 0.1 mL of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm after 10 min of incubation. The reducing power capacity was expressed as microgram of ascorbic acid equivalent per milligram of fresh weight ($\mu g \cdot mg^{-1} \cdot$AAE FW).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

To determine the antioxidant potential in terms of hydrogen donating or radical scavenging ability, DPPH radical scavenging capacity of the tissue extracts were investigated according to Blios (1958). The sample extracts (40 μL) was added to 1.960 mL of 0.01 mM methanol solution of DPPH and incubated in dark for 15 min. Then the absorbance was read at 517 nm and the scavenging activity was calculated using the following formula:

DPPH radical-scavenging activity (%) = $[(A_c-A_s)/A_c] \times 100$, where A_c denotes the absorbance of control (without sample) and A_s denotes the absorbance of sample (extract or ascorbic acid).

Analysis of Antioxidant Enzyme Activities

For antioxidant enzyme extraction, 100 mg of fresh leaf and root samples were ground in 1.5 mL ice cold 100 mM sodium phosphate buffer (pH 7.0) for guaiacol peroxidase (GPX) activity assay and 50 mM sodium phosphate buffer with 1 mM EDTA, 0.05% Triton X 100, and 2% polyvinylpyrrolidone for sodium dismutase (SOD), catalase (CAT), ascrobate peroxidase (APX), and phenylalanine ammonia lyase (PAL) activity assays. The homogenates were centrifuged at 18,000 g for 20 min at 4ºC and the supernatant was employed for enzyme activity analysis and protein estimation. The SOD activity was assayed by following the protocol of Giannopolitis and Ries (1977) by the nitro blue tetrazolium (NBT) inhibition method. Guaiacol peroxidase activity was estimated based on the amount of enzyme required for the formation of tetraguaiacol per min according to Shah et al. (2001). The activity of CAT enzyme was estimated according to the method of Cakmak and Marschner (1992). APX activity was estimated by following the protocol of Nakano and Asada (1981). PAL activity was determined according to the method outlined by Zhan et al. (2012). The total protein content of the samples was determined by the Bradford's method (Bradford, 1976). All the chemicals used for the phytochemical analysis, antioxidant potential assessment and antioxidant enzymes estimation were of analytical grade purchased from Sigma Aldrich (St. Louis, MO, USA).

Statistical Analysis

The light treatments were set up in a completely randomized design with five replications per treatment containing five explants in each container. All the assays were performed in triplicates and the results were averaged. Significant differences among the treatments were determined by analysis of variance (ANOVA) followed by Duncan multiple range tests at a significant level of 0.05 using Statistical Analysis System (SAS, V.6.12) computer package (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

In the present study the influence of light quality on the morphogenesis, phytochemicals content, and antioxidant property of *R. glutinosa* cultured in vitro were investigated. Firstly, the morphological differences among the light treatments were observed clearly (Fig. 1). Except shoot length, all the other growth traits were significantly increased by the blue LED treatment than the other light treatments (Table 1). Interestingly, shoot length in the red LED treatment increased

Table 1. Growth traits measured after 4 weeks of light treatments of *R. glutinosa* cultured in vitro.

Treatment	Shoot length (cm)	Length of the longest root (cm)	No. of roots	Leaf traits		Fresh weight (g)	Dry weight (mg)
				Number	Length (cm)		
White FL	2.7 ± 0.3 b[z]	10.9 ± 1.7 b	8.3 ± 0.5 b	4.0 ± 0.7 b	2.3 ± 0.2 b	0.2 ± 0.0 bc	20.0 ± 0.0 b
Blue LED	2.6 ± 0.5 b	12.9 ± 0.7 a	12.0 ± 0.7 a	5.8 ± 0.5 a	3.1 ± 0.1 a	0.4 ± 0.0 a	50.0 ± 0.0 a
Red LED	3.3 ± 0.6 a	7.5 ± 0.3 c	6.5 ± 0.5 c	4.3 ± 0.5 b	1.7 ± 0.1 c	0.3 ± 0.0 b	30.0 ± 0.0 b
F-test	*	***	***	**	***	*	*

Data are the mean ± SE from three replicates.
[z]Mean separation within columns by Duncan's multiple range test at $p = 0.05$.
NS,*,**,*** Nonsignificant or significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Fig. 1. In vitro *R. glutinosa* cultures under the different light treatments for 4 weeks: A, white FL; B, blue LED; and C, red LED.

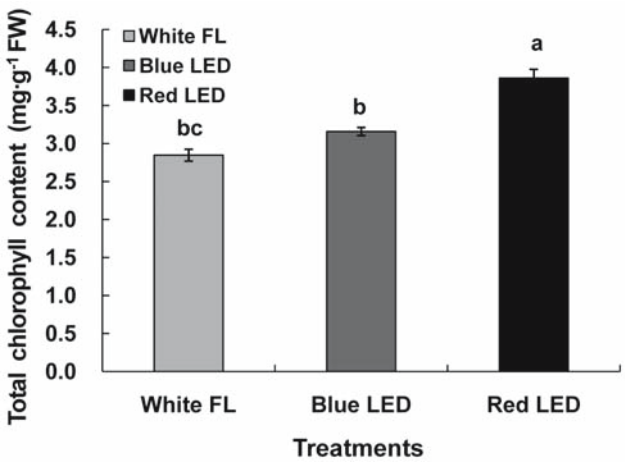

Fig. 2. Effect of light treatment on total chlorophyll content of *R. glutinosa*. Data are the mean ± SE from three replicates. Different letters in one measurement indicate statistically significant difference at $p \leq 0.05$ by Duncan multiple range test.

by 27.0 and 22.2% than in the blue LED and white FL treatment, respectively. Previous report by Hahn et al. (2000) on the effect of LED lights on *R. glutinosa* in vitro cultures also reported the increase in shoot length by red LED. Enhancement of stem elongation by red light has been widely reported in several plants such as sweet potato, chrysanthemum, and strawberry (Kim et al., 2004; Nhut et al., 2003; Sivakumar et al., 2006). According to Toyomasu et al. (1993), red light is capable to induce the endogenous gibberellins (GA) in the plant which is an important plant growth regulator involved in cell elongation and root inhibition. Furthermore, the enhancement of the GA level directly stimulated the mitosis in both apical and sub-apical meristems (Arney and Mitchell, 1969). Though not studied in this experiment, the red LED could have triggered the endogenous GA level.

Even though the red LED treatment favored the shoot elongation, the root induction was inhibited, whereas the blue LED treatment highly induced the root growth. In detail, the blue LED treatment promoted the root length by 72.0 and 18.3%, and number of roots by 84.6 and 44.5% than the red LED and white FL treatments, respectively. This is the first report documenting the effect of spectral quality on the root traits of *R. glutinosa* cultured in vitro. Similarly, Canamero et al. (2006) reported the reduction of root growth upon blue light receptor (cryptochrome)-mutation in *Arabidopsis* seedling, whereas the overexpression of cryptochrome showed significantly increased root growth than the control. Additionally, the lesser root growth observed in the red LED treatment was supported by the inhibitory activity of red light receptors (phytochromes A and B) on root elongation in comparison to dark grown seedlings (Correll and Kiss, 2005). The mechanism or pathways such as photoreceptors, auxin signaling and light piping, could have involved in the root induction (Canamero et al., 2006; Sun et al., 2005). In concordance with our results the application of blue light enhanced the rooting in *Triticum aestivum* (Dong et al., 2014).

In contrast with other treatments, the blue LED treatment improved the leaf traits such as number of leaves by 43.8 and 34.8% and leaf length by 34.5 and 86.8% respectively than white FL and red LED treatments. Generally, leaves perceive the light signals, especially blue and red, readily from the environment via photoreceptors and the modulation in the spectral quality affects the leaf characteristics (Kim et al., 2004; Park et al., 2012). In our experiment, the blue LED promoted the number of leaves more than the red LED and white FL. The increase in leaf traits allows the leaf to absorb more light which directly influences the photosynthesis for enhanced growth and development of the plant (Nishimura et al., 2009). According to Muneer et al. (2014), the blue LED promoted the photosynthesis of lettuce plants grown under controlled environment by promoting the photosystem-related proteins. Similarly, the LED treatments greatly influenced the chlorophyll synthesis in *R. glutinosa* in this study. However, the chlorophyll synthesis was markedly increased in the red LED, followed by the blue LED treatment (Fig. 2). In general, light is a primary factor important for the chlorophyll synthesis. The plant pigment synthesis is controlled by different photoreceptors that absorb light at different wavelengths (Stuefer and Huber, 1998). Similar to our result, Dong et al.

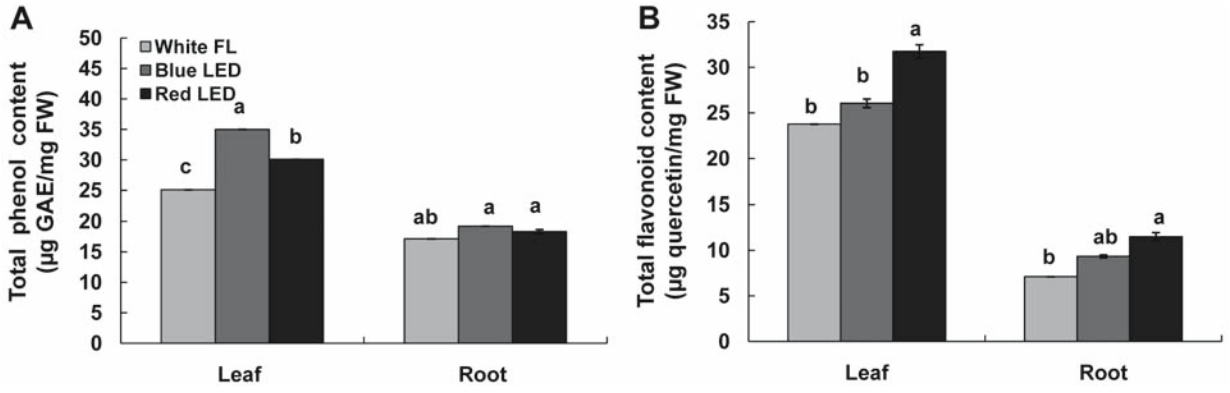

Fig. 3. Effect of light treatment on phytochemical contents of leaf and root extracts of *R. glutinosa*: A, total phenol content; and B, total flavonoid content. Data are the mean ± SE from three replicates. Different letters in one measurement indicate statistically significant difference at $p \leq 0.05$ by Duncan multiple range test.

(2014) demonstrated the increase in chlorophyll content by red light exposure. Furthermore, biomass of *R. glutinosa* treated with the blue LED significantly increased i.e., fresh weight by 91.3% than the white FL and 57.1% than the red LED (Table 1). In a similar fashion with fresh weight, dry weight in the blue LED increased by 150 and 66.0% than the control and the red LED treatment, respectively. Concordantly, Wheeler et al. (1991) also suggested the effectiveness of the blue and red light sources for healthy plant growth. The improvement of fresh and dry weights by the blue or red LED treatment alone or in combination was reported in *Oncidium* (Mengxi et al., 2011). Overall our growth traits results suggest that the blue LED can be incorporated during the in vitro growth for improved plant production.

In the present study, spectral qualities not only affected the plant growth but also augmented the phytochemicals having both defense and potential pharmaceutical values such as total phenol and total flavonoid in both leaf and root tissues (Fig. 3). The greatest total phenol contents were observed in the leaf extracts of *R. glutinosa* in the blue LED treatment (35 ± 0.05 μg GAE/mg) (Fig. 3A). Interestingly, the blue LED significantly increased the total phenol contents of the leaf extracts by 39.3% than the white FL and 17.7% than the red LED treatment. However, neither the blue nor the red LED treatment led to significant enhancement of total phenol in the root extracts. In general, the occurrences of higher phenolic contents enhance the antioxidant property of the plant because, phenols possess ideal structural properties for scavenging free radicals and thus it prevents from oxidative damages (Reddy et al., 2012). On the other hand, the total flavonoid contents were enhanced by the red LED treatment (Fig. 3B). In detail, the red LED treatment increased the total flavonoid contents of the leaf extract by 33.6 and 21.83% and root extracts by 61.7 and 23.1%, respectively, than the white FL and blue LED treatments. Similar to our results, the ability of the red light to induce the production of flavonoid (quercetin) complexes in *Pisum sativum* either by phytochrome-mediated or stress-mediated activation of flavonoid synthesis has been reported by Bottomley et al. (1966). Flavonoids are also known to play an important roles against light-induced damages (Jaakola et al., 2004), perhaps the same happened to induce the flavonoid accumulation in *R. glutinosa* cultured in vitro in the LED treatments in this study.

Furthermore, the antioxidant capacity of the root and leaf extracts of *R. glutinosa* grown under different light qualities were determined and confirmed by several assays. Firstly, the ability of the extracts to reduce the molybdate from Mo (IV) to Mo (V) was assessed by the phosphomolybdenum assay. The greatest total antioxidant capacity of the leaf and root extracts was observed in the blue LED treatment, followed by the red LED treatment (Fig. 4A), which might be due to the higher content of total phenol in the blue LED and red LED treatment. These results were concordant with the previous report, demonstrating the presences of highest total antioxidant capacity in *R. glutinosa* leaf extracts (Piatczak et al., 2014).

Secondly, in a reducing power assay, the reduction of potassium ferric cyanide, expressed in ascorbic acid equivalents, significantly increased upon the addition of the tissue extracts. The higher values were observed in the root extracts than in leaf extracts, especially in the red LED, followed by the blue LED treatment (Fig. 4B). This particular assay is considered as one of the robust methods for the determination of the presence of a wide range of antioxidants in plant extracts (Moein et al., 2008). In accordance to our results, Jeong et al. (2013) documented the highest reducing power potential in the root extract of *R. glutinosa*. The enhanced reducing power of the roots might be possibly due to the presence of several important iridoid glycosides (Chung et al., 2006). Furthermore, the occurrence of higher content of flavonoid was also responsible for the increased reducing power activity

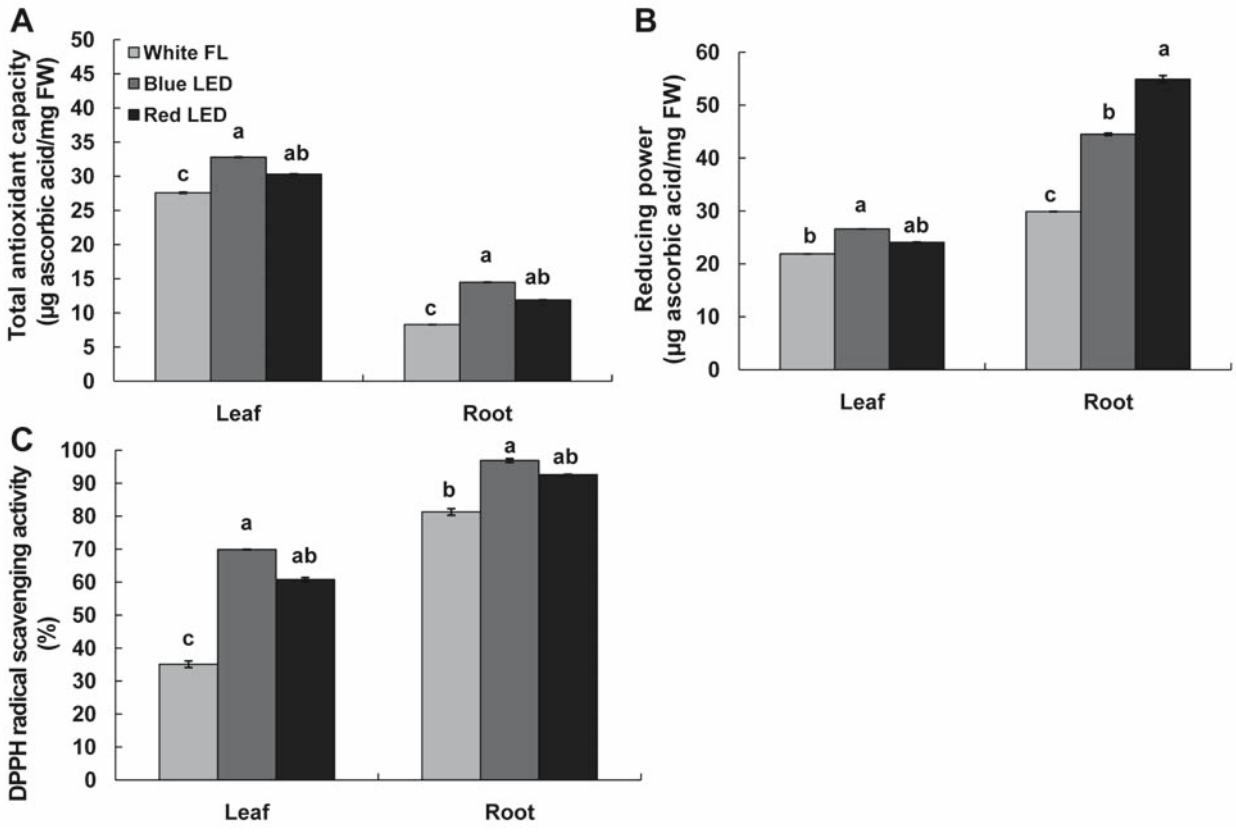

Fig. 4. Effect of light treatment on free radical scavenging and antioxidant activities of the leaf and root extracts of *R. glutinosa*: A, total antioxidant capacity (phosphomolybdenum reduction capacity); B, reducing power; and C, DPPH radical scavenging activity. Data are the mean ± SE from three replicates. Different letters in one measurement indicate statistically significant difference at $p \leq 0.05$ by Duncan multiple range test.

in the root extracts in the red LED treatment. Thus, it can be clearly noted that the increase in total phenol and flavonoid contents by the LED treatments has a strong positive correlation with the enhancement of antioxidant capacity (Table 2).

Moreover, the antioxidant activity assessment by DPPH radical scavenging also revealed the greater DPPH scavenging ability of the root extracts compared to leaf extracts (Fig. 4C). Among the light treatments the highest amount of DPPH radicals was scavenged by the blue LED-treated root extract which was followed by the red LED treatment. Apparently, the occurrence of higher contents of phytochemicals in the blue and red LED treatments positively affected the DPPH radical scavenging activity of the root extract. The variation in the antioxidant capacity and radical scavenging potential of the leaf and root extracts upon light treatments could be attributed to the differential accumulations of an individual secondary metabolite in the tissues as well as the nature of the assay. However, in all antioxidant activity assays the least amount of antioxidant activity was displayed in the white FL treatment. In concordance with Samuoliene et al. (2012), the immense rise on the protective bioactive compounds upon the LED treatments led to the significant improvement of antioxidant property and free radical scavenging potential of *R. glutinosa*.

Furthermore, the modulation of light quality significantly enhanced the activities of antioxidant enzymes (Fig. 5). In general the increase in ROS production triggers the activities of antioxidant enzymes to prevent the cell damage due to oxidative stress. The activity of SOD increased in both the leaf and root extracts upon the blue LED treatment (Fig. 5A). According to Dewir et al. (2006), the SOD, commonly called as metalloenzymes, acts as the first line of defense against the oxidative stress and dismutase the highly reactive superoxide radical into hydrogen peroxide and oxygen molecules. Similarly the GPX activity also increased in the blue LED-treated leaf and root extracts (Fig. 5B). The increased activity of the GPX could be associated with the increased lipid peroxidation level (Shah et al., 2001), whereas higher APX activity was noted in the leaf extracts in the blue LED treatment compared to root extract (Fig. 5C). Generally, the APX is predominantly required to scavenge the harmful H_2O_2 produced in the chloroplast. It also acts as an indispensable component of the ascorbate-glutathione cycle to scavenge the harmful H_2O_2 by utilizing the reducing power

Table 2. Pearson correlation coefficients of phytochemicals and antioxidant property of *R. glutinosa.*

Tissue	Light treatment	Phytochemicals and total antioxidant capacity	Correlation coefficient of total antioxidant capacity (r^2)	Correlation coefficient of reducing power property (r^2)
Leaf	White FL	Total phenol	0.92*	0.98**
		Total flavonoid	0.95**	0.99**
		Total antioxidant capacity	-	0.98**
	Blue LED	Total phenol	0.98**	0.89**
		Total flavonoid	0.87*	0.70
		Total antioxidant capacity	-	0.96**
	Red LED	Total phenol	0.98**	1.00**
		Total flavonoid	0.92*	0.84*
		Total antioxidant capacity	-	0.98**
Root	White FL	Total phenol	0.78	0.84*
		Total flavonoid	0.84*	0.78
		Total antioxidant capacity	-	1.00**
	Blue LED	Total phenol	0.96**	0.99**
		Total flavonoid	0.97**	0.98**
		Total antioxidant capacity	-	0.99**
	Red LED	Total phenol	0.99**	0.99**
		Total flavonoid	0.98**	0.99**
		Total antioxidant capacity	-	0.97**

*,**Correlation significant at $p \leq 0.05$ and 0.01, respectively.

property of ascorbic acid (Nakano and Asada, 1981). Subsequently the CAT enzyme, a universal oxido-reductase, was enhanced by the blue LED treatment in both the leaf and root extracts (Fig. 5D). The CAT readily scavenges the excess ROS by the reduction of H_2O_2 to water and molecular oxygen (Lin and Kao, 2000). Moreover, the PAL activity increased upon either blue or red LED illumination in the leaf and root extracts (Fig. 5E). The PAL catalyzes the most important step in the phenylpropanoid pathway by which the polyphenolic compounds are synthesized. Interestingly, the transcriptional regulation of the PAL can be activated by light and consequently induce the phenol synthesis (Zhan et al., 2012). The PAL activity can be attributed to the augmentation of the phytochemicals in the LED treatments. Similarly, Xu and Davey (1983) noted the increase in PAL activity along with other intermediates in the phenylpropanoid pathway upon blue light irradiation onto strawberry fruits. In concordance with our results, the enhanced antioxidant enzyme activities by the LED treatments were also observed in *Oncidium* by Mengxi et al. (2011). However, none of the antioxidant enzyme activities were elevated by the control white FL. Hence, the LED light sources can be employed during the micropropagation process to enrich the antioxidant activity of *R. glutinosa*. In addition the greatest total protein contents were observed upon the LED treatment especially on the blue LED treatment (Fig. 5F). The improvement of protein synthesis and enzyme activity can be triggered by the LED treatments than white FL treatment (Mengxi et al., 2011). In the future the possible molecular regulations of antioxidant enzymes by the blue and red LEDs need to be studied in depth. Consequently, our results suggest that the blue or red LED treatments have the capacity to enhance the antioxidant defense mechanism and to elicit the accumulation of potential secondary metabolites in *R. glutinosa* cultured in vitro.

In conclusion, we have demonstrated the influence of the blue and/or red LEDs on the growth traits, accumulation of secondary metabolites, antioxidant potentials, and antioxidant enzyme activities of *R. glutinosa* grown under an in vitro condition. Our results suggest the blue or red LEDs are the most appealing light sources for the in vitro propagation of *R. glutinosa.* It is worth to note that influence of the LEDs on metabolites and antioxidant capacity improves the ROS scavenging ability of *R. glutinosa*, as an important medicinal plant will be a major breakthrough on pharmaceutical applications.

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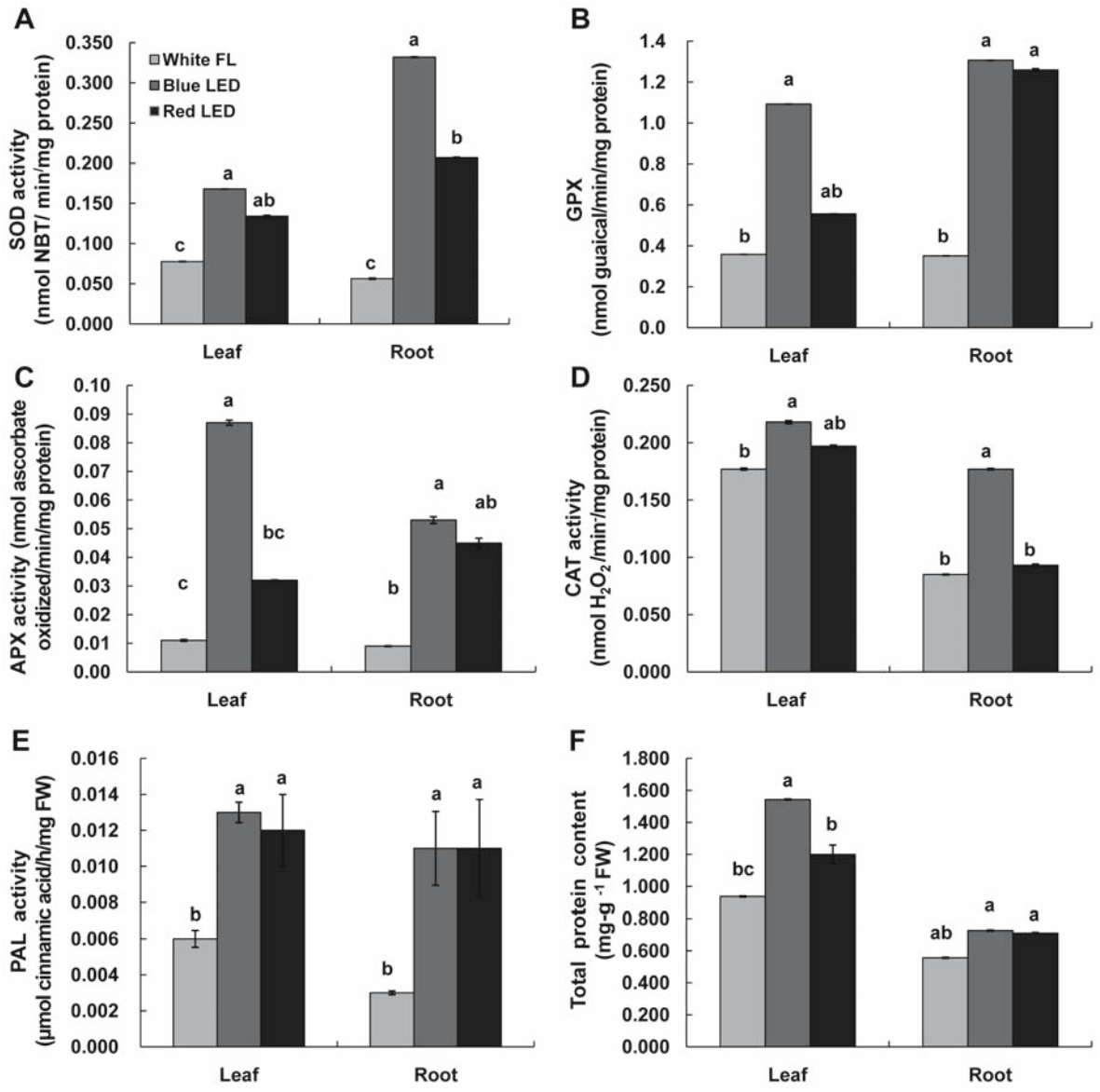

Fig. 5. Effect of light treatment on antioxidant enzyme activity and total protein content of the leaf and root extracts of *R. glutinosa*: A, superoxide dismutase (SOD); B, guaiacol peroxidase (GPX); C, ascorbate peroxidase (APX); D, catalase (CAT); E, phenylalanine ammonia lyase (PAL), and F, total protein content. Data are the mean ± SE from three replicates. Different letters in one measurement indicate statistically significant difference at $p \leq 0.05$ by Duncan multiple range test.

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