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Research Article

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Phenylalanine ammonia-lyase expression and pyranocoumarin accumulation in *Angelica gigas* plantlets exposed to light-emitting diodes

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ABSTRACT

Angelica gigas (Dang Gui) is an important medicinal plant. In this study, we examined the accumulation of pyranocoumarin (decursin and decursinol angelate) and the expression of phenylalanine ammonia-lyase (*PAL*) in Korean angelica plantlet grown under different light-emitting diodes (LEDs) (red, orange, green, blue, and white). Three weeks after LED exposure (WAE), the transcript levels of phenylalanine ammonia-lyase mRNA in seedlings grown under orange LEDs were 4-, 18-, and 7-fold higher than those in seedlings grown under green, blue, and white LEDs, respectively. The decursinol angelate content was almost double than the decursin content. The highest levels of decursin (3.2 mg/g dry weight) and decursinol angelate (6 mg/g dry weight) were detected in plants grown under orange LEDs, at 2 WAE. Therefore, we suggest that orange LEDs may affect decursin and decursinol angelate accumulation. The findings of this study could help to determine an effective strategy for producing secondary metabolites in *A. gigas* using LED technology.

KEYWORDS: *Angelica gigas*, decursin, decursinol angelate, light-emitting diodes, phenylalanine ammonia-lyase, transcription

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INTRODUCTİON

Angelica gigas (Dang Gui), an important medicinal plant, has been widely used in traditional Korean medicine. This plant, known as Korean angelica, has dark purple flowers, whereas Chinese and Japanese angelica flowers (*A. sinensis* and *A. acutiloba*, respectively) are white (Ahn *et al*., 2008). (Lee *et al*., 2003a) and (Kim *et al*., 2008) reported that *A. gigas* roots have antioxidant activity, skin-whitening effects, and ultraviolet (UV)-protective effects. In addition, it is documented that *A. sinensis* has also been widely used in China for the remedy of several diseases, i.e. anemia, asthma, cardiovascular diseases, chronic bronchitis, hypertension, and rheumatic disorder (Lin *et al*., 1998; Lao *et al*., 2004; Lu *et al*., 2004). The major functional compounds in this plant are coumarins of decursin, decursinol, and nodakenin, and a variety of secondary metabolites are biosynthesized in *A. sinensis*. Additionally, decursin and decursinol angelate have been involved in anti-androgen receptor signaling, cytotoxicity, and neuroprotective activities (Konoshima *et al*., 1968; Pachaly *et al*., 1996; Lee *et al*., 2003b).

The quality and quantity of chemical compounds of medicinal plants are affected by climatic and edaphic factors such as temperature, duration of sun exposure, precipitation, soil, and exposure to light-emitting diodes (LEDs) (Kim *et al*., 2011; Tuan *et al*., 2013; Thwe *et al*., 2014). Among these factors, LEDs have many benefits for facilitating growth within regulated environments. These advantages comprise wavelength specificity, adjustable light intensity, high energy-conversion efficiency, longer life, and low thermal energy output (Okamoto *et al*., 1996; Schuerger *et al*., 1997). Red light can enhance starch content by reducing the translocation of photosynthates from

the leaves (Sæbø *et al.*, 1995), whereas blue light is crucial for the development of chloroplast, chlorophyll synthesis, and stomata opening (Senger, 1982). Blue light has been shown to play a significant role in a broad range of plant processes, including photomorphogenesis, photosynthesis, phototropism, and stomatal opening (Staiger, 2008). Our group reported that red and blue lights cause substantial increases in catechin and rutin contents, respectively, in *Fagopyrum tataricum* sprouts (Thwe *et al.*, 2014). The total carotenoid content of Tartary buckwheat sprouts grown under white LED was higher than that of sprouts grown under blue and red LEDs (Tuan *et al.*, 2013).

Moreover, in a previous study from our laboratory, we reported that the expression level of genes involved in the anthocyanin biosynthetic pathway and anthocyanin accumulation in *F. tataricum* sprouts grown under light or dark conditions were significantly influenced (Li *et al.*, 2012). The phenylpropanoid pathway plays a vital role in the production of secondary metabolic compounds in plants and acts as an intermediate for the production of various metabolites (Dixon and Paiva, 1995) (Figure 1). Phenylalanine ammonia-lyase (PAL) is one of the most key regulatory enzymes in the phenylpropanoid biosynthetic pathway which converts L-phenylalanine to trans-cinnamic acid by catalyzation process (Liu *et al.*, 2006). In plants, the PAL gene has been extensively studied due to its significance in the production of numerous secondary metabolites. In A. *gigas*, PAL plays a vital role in the decursin and decursinol angelate biosynthetic pathway (Park *et al.*, 2010).

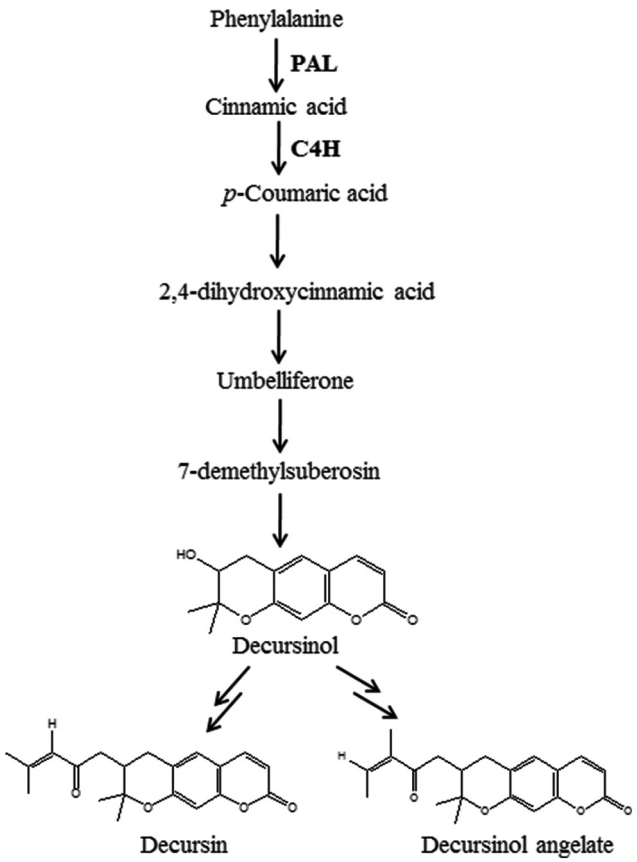

Figure 1: Schematic view of decursin and decursinol angelate biosynthetic pathway in *Angelica gigas*. PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase

MATERIALS AND METHODS

Plant Materials and Growth Conditions

A. *gigas* seeds were harvested in the fields of Youngju Agriculture Technical Center in March 2014. The seeds were washed for three days with tap water and transferred into pots filled with perlite-mixed soil. The seedlings were cultured in a growth chamber maintaining a flux rate of 120 μmol m^{-2} s^{-1}, at 22–24°C and 70% humidity. After 5 weeks following germination, seedlings were exposed to different LEDs of red (SL5-RT501T^{-1}, 625 nm), orange (SL5-YT501T^{-1}, 590 nm), green (SL5-SG501TZ, 525 nm), blue (SL5-SB501T^{-Z}, 467 nm), and white (SL5-SW501T^{-1}, 380 nm) for 3weeks. LEDs light intensities were maintained at 72 ± 3 μmol m^{-2} s^{-1}. The seedlings were harvested at one, two, and three WAE. Harvested samples were immediately frozen in liquid nitrogen and then the samples were stored at −80 °C.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

RNA was extracted from each Korean angelica seedling using a Plant Total RNA Mini kit (Geneaid, Taiwan). For cDNA synthesis, the ReverTra Ace-α- Kit (Toyobo, Japan) was used according to the manufacturer's instructions. The cDNA was diluted 20 times with RNase-free water for qRT-PCR. Transcript levels of *AgPAL and AgC4H* genes were analyzed by qRT-PCR (Bio-Rad, Hercules, CA) using a 2× Real-Time PCR Smart mix (SolGent, Korea). Gene-specific primer sets were designed for qRT-PCR as follows: *AgPAL*- (forward: 5'-AAC AGC ACA ATC AAG ATG TGA ACT CC-3'; reverse: 5'-AAT TCT CCT CCA AAT GCC TCA AGT C-3') and *Ag18S*- (forward: 5'-CTT AGT TGG TGG AGC GAT TTG TCT G-3'; reverse 5'-ACC TGT TAT TGC CTC AAA CTT CCG T-3'). qRT-PCR was conducted using a 2× Real-Time PCR Smart mix (SolGent, Korea). The qRT-PCR cyclic condition was as follows: 3 min at 95°C followed by 40 cycles of 10 s at 95 °C, 10s at 55 °C s, and 30 s at 72 °C. All the reactions were carried out in triplicates. The housekeeping gene 18S ribosomal gene (Accession number DQ647697) was used as an internal control.

High-Performance Liquid Chromatography (HPLC) Analysis

Extraction and analysis of decursin and decursinol angelate was analyzed based on the protocol described by Park *et al.* (Park *et al.*, 2010) with slight modification. Dried (500 mg) fine powder samples were mixed with 70% ethanol (30 mL) and incubated for 1h in a water bath at 50 °C. The mixture was centrifuged at 10,000 rpm for 15 min. Twenty-five milliliter of supernatant was concentrated by using a vacuum concentrator and then extracted with dichloromethane three times. Then this mixture was dried under vacuum, and add 1 ml acetonitrile. These mixtures were passed through a 0.45 μm PTFE filter. The separation of compounds was carried out using a Futecs

model of NS-4000 HPLC system (Daejeon, Korea) equipped with a reversed-phase OptimaPak C18 (5 µm, 250 mm × 4.6 mm) column and UV detector. The sample injection volume was 10 µL. The mobile phase used in this system was 40% acetonitrile, 50% water, and 10% tetrahydrofuran. The flow rate of the solvent was 0.8 mL/min and the column temperature was 35 °C, and the chromatogram was acquired at 280 nm. Decursin and decursinol angelates were identified based on their HPLC peak area ratios and quantified based on the retention time, peak areas, and response factor with the respective external standards.

Statistical Analysis

All data were evaluated using Statistical Analysis System (SAS version 9.2, Cary, NC, USA). The mean and standard deviation were calculated from three biological replicates. The significant differences among the means were calculated by using Duncan Multiple Range Test.

RESULTS AND DİSCUSSİON

In this study, pyranocoumarin (decursin and decursinol angelate) accumulation and Phenylalanine ammonia-lyase (PAL) expression were analyzed in Korean angelica seedlings grown under different LED lights. The study aimed to examine the growth response of Korean angelica seedlings under different LED lights (red, blue, orange, green, and white). In addition, for better understanding, the regulation of genes engaged in the phenylpropanoid biosynthetic pathway, especially of PAL. Moreover, we had analyzed the pyranocoumarin accumulation in A. *gigas* plantlets in response to LEDs.

A. *gigas* seedlings were grown under different light conditions, i.e. white, blue, orange, green, and red LEDs for three days and the changes in transcript levels of *AgPAL* were measured at 1-week intervals using qRT-PCR (Figure 2). The gene expression levels in seedlings grown under green, blue, and white lights were almost similar, whereas those of seedlings grown under red and orange LEDs were dissimilar. Specifically, *AgPAL* transcript levels in plants grown under red and orange LEDs were the highest at 3 WAE, whereas they were highest in plants irradiated under green, blue, and white LEDs at 2 WAE. Transcript levels gradually increased from 1 to 3 WAE in plants grown under orange LEDs. The expression levels of *AgPAL* in seedlings grown under orange LEDs were 4-, 18-, and 7-fold higher than those in seedlings raised under green, blue, and white LEDs, respectively, at 3 WAE. Moreover, the transcript levels of *AgPAL* in plants grown under orange LEDs were 70- and 2-fold higher at 3 WAE than at 1 and 2 WAE, respectively. For plants grown under red LEDs, *AgPAL* expression levels were 9-fold greater at 2 and 3 WAE than at 1 WAE. Finally, for plants grown under green LEDs, *AgPAL* expression levels were 10- and 2-fold higher at 2 WAE than at 1 and 3 WAE, respectively. Recently, our group reported that the expression of most genes involved in flavonoid biosynthesis was observed on day two after LED exposure, particularly for *FtPAL* and*FtF3′H*, showing greater expression in Tartary buckwheat sprouts grown under blue and white than in those grown under red LED (Thwe *et al.*, 2014).

Most of the carotenoid biosynthetic pathway genes in Tartary buckwheat sprouts grown under white LEDs expressed higher transcript levels at day eight after sowing compared with gene expression in plants raised under blue and red LEDs conditions (Tuan *et al.*, 2013). However, in this study, the expression level

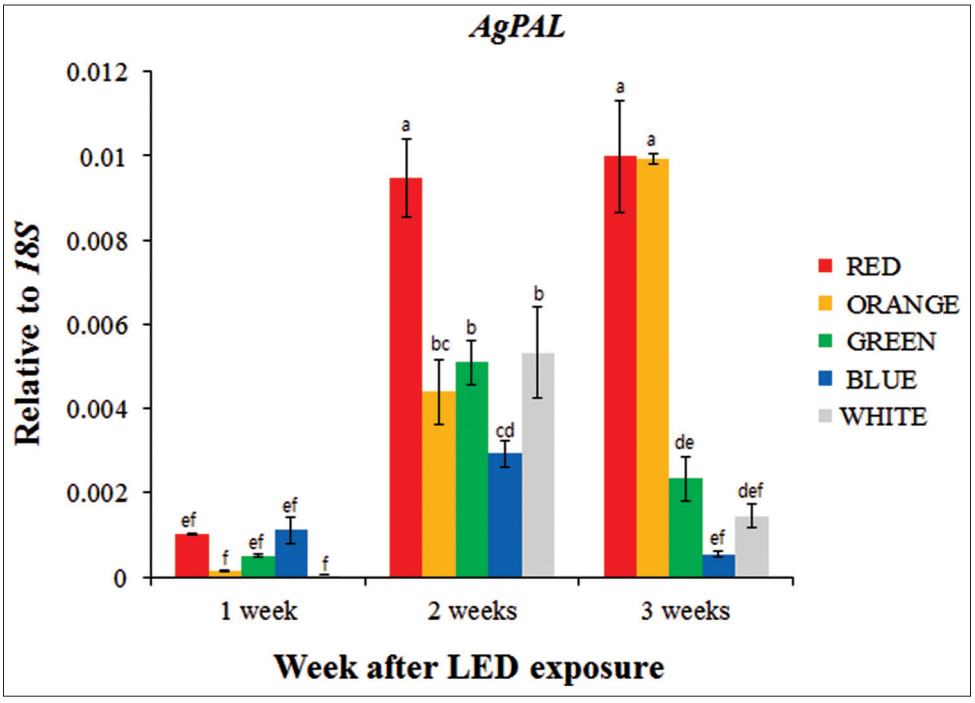

Figure 2: Variation of transcript levels of AgPAL in seedlings grown under LED lights in *A. gigas*. The values of transcript levels were determined using three biological replicates and analyzed relative to those of 18S

of *AgPAL* in the seedlings of Tartary buckwheat under red LEDs was greater than that of seedlings grown under blue and white lights. Thus, our data proposed that the expression level of *AgPAL* was dependent on the plant and the LED condition. Martín-Cabrejas *et al.*, 2003 found that light can induce metabolic changes during germination. In addition, Archetti *et al.*, 2009 and Karageorgou *et al.*, 2008 reported that metabolite changes under light conditions may occur because of the accumulation of anthocyanins, which are vacuolar flavonoids, and that anthocyanin content is positively correlated with phenolics in several species.

Previously, we reported that the transcription of both *AgPAL* and *AgC4H* has highly enhanced with 300 μM methyl jasmonate for 6 or 12 h, respectively, and that these transcripts were highly expressed in the *A. gigas* roots (Park *et al.*, 2010). In addition, the transcript levels of *AgPAL* and *AgC4H* showed a similar pattern. However, in the current study, transcription patterns of *AgPAL* varied. Unlike *AgPAL* showed very low transcription compared with Ag18S (data not shown). Finally, previous studies have primarily used only red, blue, and white LEDs. In the current study, we used green and orange LEDs in addition to red, blue, and white LEDs. Interestingly, unlike the results of other studies, we also found that red and orange LEDs had substantial effects on the transcription of *AgPAL*.

Next, we analyzed the accumulation of decursin and decursinol angelates during *A. gigas* seedling development by HPLC (Figure 3). The decursinol angelate content was two-fold higher

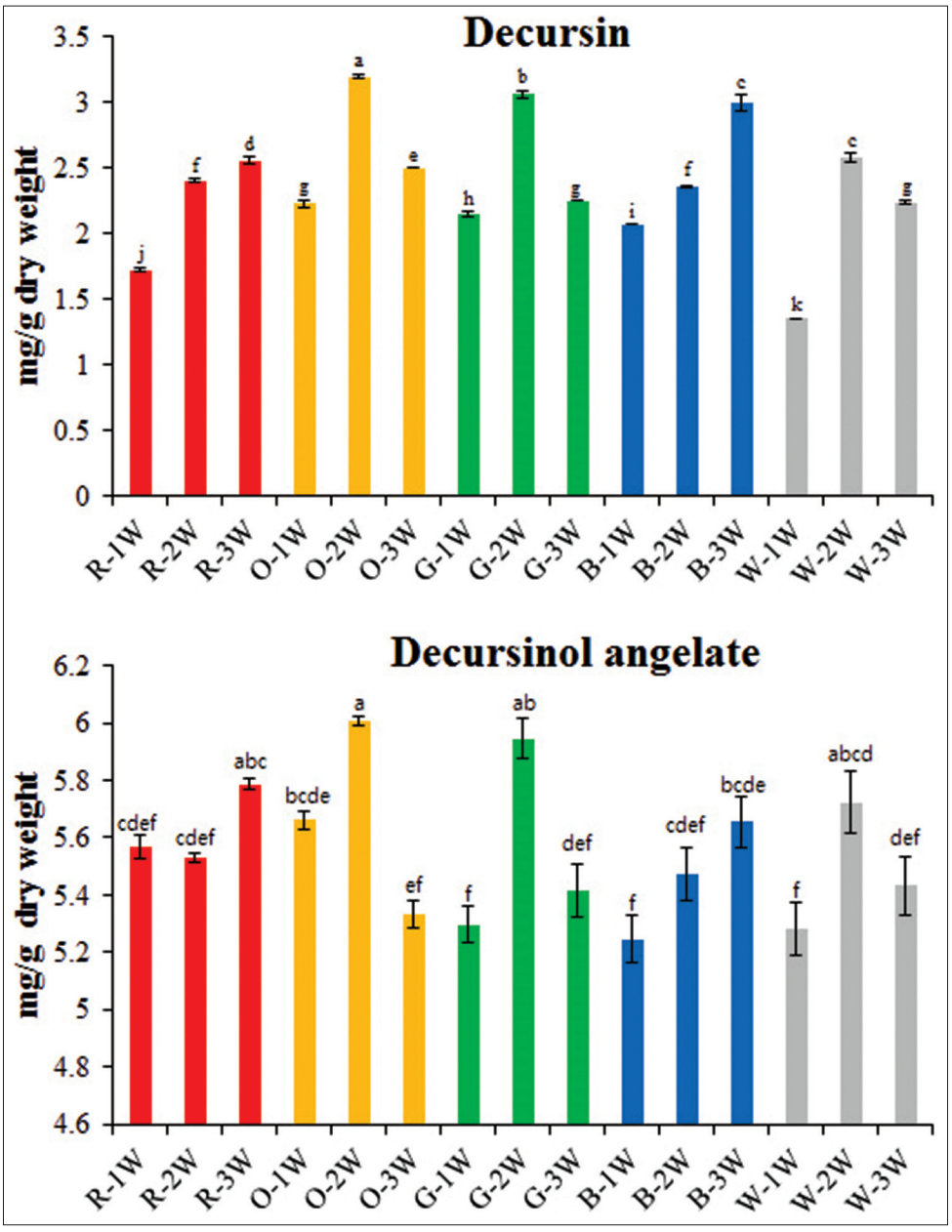

Figure 3: Analysis of decursin and decursinol angelate in seedlings grown under LED lights in *A. gigas*. The symbols R, O, G, B, and W represent red, orange, green, blue, and white, respectively; 1W, 1 week; 2W, 2 weeks; 3W, 3 weeks

than that of decursin content. Additionally, there was a significant difference in decursin and decursinol angelate accumulation at different sampling times. The highest decursin content was detected in plants grown under orange LEDs at 2 WAE (3.2 mg/g DW), whereas the lowest decursin content was observed in plants grown under white LEDs at 1 WAE (1.4 mg/g DW). Interestingly, *AgPAL* showed the highest expression in seedlings grown under orange LEDs at 3 WAE. The decursin content of seedlings grown under red and blue LEDs gradually increased from 1 to 3 WAE, whereas that in seedlings grown under orange, green, and white LEDs was the highest at 2 WAE.

In general, the light changes the intensity of the pigment by interceding the expression of genes involved in the anthocyanin biosynthetic pathway (Sheoran *et al*., 2006). Anthocyanin, flavonols, and phenolic acids are induced rapidly by irradiation, whereas dihydrochalcones, flavanols, and procyanidins do not vary in mature versus ripe apple fruits (Bakhshi and Arakawa, 2006). It is reported that the epicatechin content of T8 Tartary buckwheat was affected by light, suggesting that the effect of light on catechin and epicatechin content may depend on the cultivar of Tartary buckwheat (Kim *et al*., 2014). In addition, Thwe *et al*., 2014 found that LEDs and fluorescent lights have comparable effects on rutin content in sprouts of Tartary buckwheat. Blue light increases the accumulation of anthocyanins, which play a significant role in antioxidant activity (Duan *et al*., 2007).

CONCLUSİON

In the present study, decursin and decursinol angelate content were the highest under orange LEDs. Consistent with this, *AgPAL* mRNA levels were highest in plants grown under orange LEDs at 3 WAE. Therefore, we suggest that orange LEDs may affect decursin and decursinol angelate accumulation through PAL. Furthermore, we speculate that the findings of this study will help to establish an effective procedure for the accumulation of secondary metabolites in *A. gigas* using LED technology.

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AUTHOR'S CONTRIBUTIONS

J.S.P. and S.U.P. designed the experiments and analyzed the data. Y.B.K., W.T.P., R.S., S.K.Y., G.I.L., performed the experiments and analyzed the data. Y.B.K., J.S.P., and S.U.P. wrote the manuscript. All authors read and approved the final manuscript.

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