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Effects of White, Blue, and Red Light-Emitting Diodes on Carotenoid Biosynthetic Gene Expression Levels and Carotenoid Accumulation in Sprouts of Tartary Buckwheat (*Fagopyrum tataricum* Gaertn.)

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ABSTRACT: In this study, the optimum wavelengths of light required for carotenoid biosynthesis were determined by investigating the expression levels of carotenoid biosynthetic genes and carotenoid accumulation in sprouts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) exposed to white, blue, and red light-emitting diodes (LEDs). Most carotenoid biosynthetic genes showed higher expression in sprouts irradiated with white light at 8 days after sowing than in those irradiated with blue and red lights. The dominant carotenoids in tartary buckwheat sprouts were lutein and β-carotene. The richest accumulation of total carotenoids was observed in sprouts grown under white light (1282.63 μg g^{-1} dry weight), which was relatively higher than that in sprouts grown under blue and red lights (940.86 and 985.54 μg g^{-1}, respectively). This study might establish an effective strategy for maximizing the production of carotenoids and other important secondary metabolites in tartary buckwheat sprouts by using LED technology.

KEYWORDS: *carotenoids, gene characterization, light-emitting diode, sprout, tartary buckwheat*

INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.), belonging to the Polygonaceae family, is mainly distributed in the Himalayan region of Nepal, China, Bhutan, and India and has long been used as a daily food and traditional medicine.[1,2] It is rich in proteins, fiber, vitamins, phenylpropanoid glycosides, and multiple flavonoids[3−5] and has been reported to possess various beneficial pharmacological effects, including anticancer, antidiabetic, and antioxidant properties.[6,7] Recently, tartary buckwheat was considered as a new functional food because of its benefits for human health. It has been used to develop many commercial products such as noodles, herb tea, and crackers. In addition, seed sprouts of tartary buckwheat have been popular in the international market because of their excellent dietary sources of nutrients, amino acids, minerals, carotenoids, and phenolic compounds.[8,9]

Carotenoids, consisting of 40 carbon molecules, are the second largest pigment group in nature, with over 700 members identified to date.[10] In plants, carotenoids serve as pigments that impart to flowers and fruits yellow, orange, and red colors to attract pollinators and agents of seed dispersal.[11] Carotenoids play various essential functions in many physiological processes of plants, such as harvesting light for photosynthesis, protecting the photosystem from photo-oxidation, and stabilization of lipid membranes.[12−14] In humans, carotenoids are essential nutrients and health-promoting compounds that are not synthesized de novo and need to be acquired from the diet. Carotenoids are the primary precursors of vitamin A, which is one of the most important micronutrients that affect the health of humans.[15] Vitamin A deficiency, which increases the risk of infectious disease, particularly measles, diarrhea, and malaria, is considered as the most common public health problem among preschool children.[16,17] Epidemiological studies have suggested that a diet containing carotenoid-rich vegetables and fruits plays a role in reducing the risk of cancer, cardiovascular disease, macular degeneration, cataracts, and ultraviolet-induced skin damage.[18−21]

Because of the importance of carotenoids for plant and human health, the carotenoid biosynthesis pathway has been well studied in higher plants for several decades (Figure 1).[22,23] Molecular characterization of all genes related to the carotenoid biosynthetic pathway has allowed the enhancement of carotenoid accumulation in plants. Transgenic Golden Rice I and II are two excellent examples for metabolic engineering of carotenoid biosynthesis in plants.[24] Besides, many studies have focused on developing efficient strategies for optimizing the

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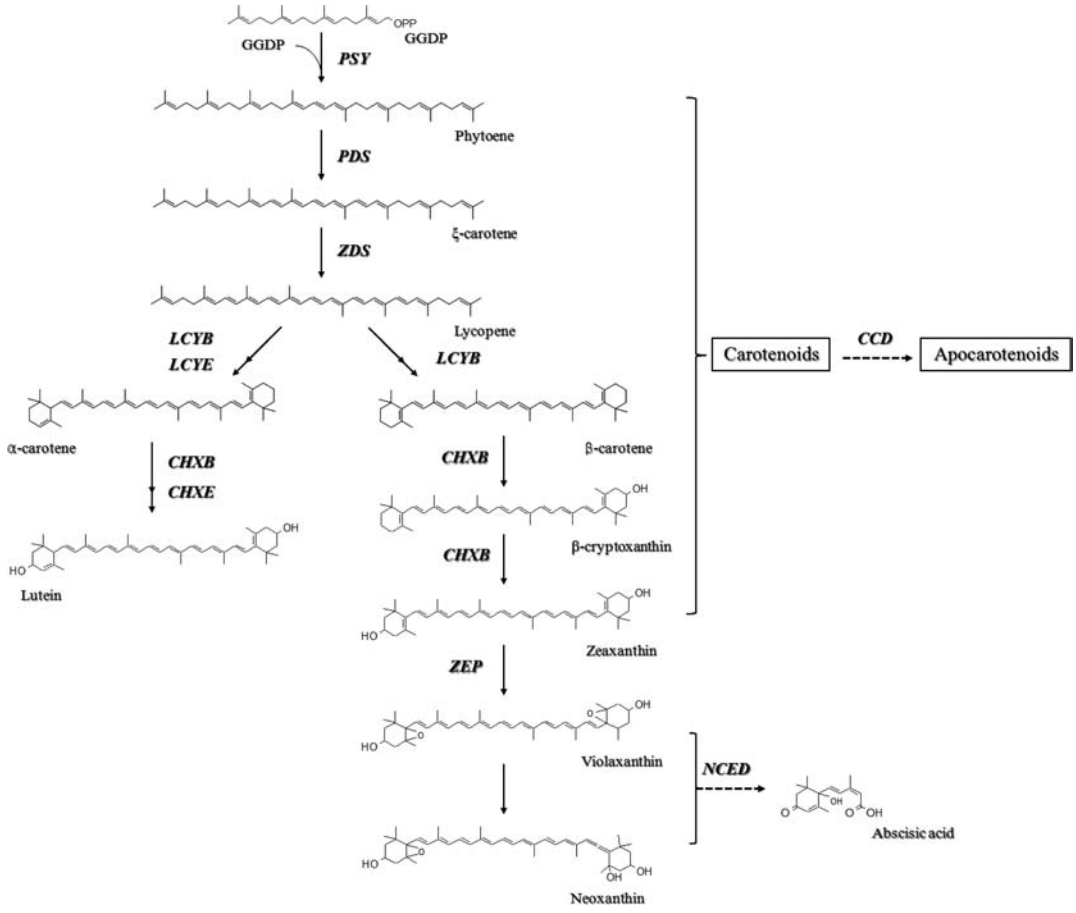

Figure 1. Carotenoid biosynthetic pathway in plants. GGDP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ξ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CHXB, β-ring carotene hydroxylase; CHXE, ε-ring carotene hydroxylase; ZEP, zeaxanthin epoxidase; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis* epoxycarotenoid dioxygenase.

production of carotenoids in plants without gene modification or breeding. Among them, application of different wavelengths of light has been reported to efficiently affect carotenoid biosynthesis in plants. The accumulation of β-cryptoxanthin in the flavedo of citrus fruits was induced by a red light-emitting diode (LED), whereas it was not affected by blue LED light.[25] The β-carotene contents of pea seedlings exposed to red LED light was significantly higher than those of seedlings exposed other lights.[26] The content of lutein in kale maximized at a wavelength of 640 nm, whereas the highest β-carotene accumulation occurred under 440 nm light treatment.[27]

In this study, we aimed to establish the optimum wavelengths of light for carotenoid biosynthesis in sprouts of tartary buckwheat. The expression levels of 10 carotenoid biosynthetic genes, namely, phytoene synthase (FtPSY), phytoene desaturase (FtPDS), ξ-carotene desaturase (FtZDS), lycopene β-cyclase (FtLCYB), lycopene ε-cyclase (FtLCYE), β-ring carotene hydroxylase (FtCHXB), ε-ring carotene hydroxylase (FtCHXE), zeaxanthin epoxidase (FtZEP), carotenoid cleavage dioxygenase (FtCCD1), and 9-*cis*-epoxycarotenoid dioxygenase (FtNCED), and carotenoid accumulation were analyzed in sprouts of tartary buckwheat under white, blue, or red LED treatments by using quantitative real-time polymerase chain reaction (PCR) and high-performance liquid chromatography (HPLC), respectively.

MATERIALS AND METHODS

Plant Materials and LED Treatments. Tartary buckwheat (*F. tataricum* Gaertn.) cultivar 'Hokkai T8' was bred by the Hokkaido Agricultural Research Center (Hokkaido, Japan). For sprouts, seeds of 'Hokkai T8' were soaked in water for 12 h before sowing. Seeds were sown in plastic pots containing vermiculite soil and grown in a growth chamber at 25 °C under high-intensity irradiation with white (wavelength, 380 nm), blue (wavelength, 470 nm), or red (wavelength, 660 nm) LEDs at a flux rate of 50 μmol s^{-1} m^{-2} for a 16 h photoperiod. The sprouts were collected at 2, 4, 6, 8, and 10 days after sowing (DAS). The experiments were repeated three times, and the mixture of three independent replicate sprouts was used for further analysis of gene expression and carotenoid accumulation. All sprout samples were frozen in liquid nitrogen immediately after harvesting and were stored at −80 °C and/or freeze-dried until RNA isolation and/or HPLC analysis.

RNA Isolation and cDNA Synthesis. Tartary buckwheat sprout samples were ground to a powdered form in a mortar by using liquid nitrogen, and total RNA was isolated using the method described by Tuan et al.[9] The quality and concentration of the total extracted RNA were determined using 1% agarose gel electrophoresis and spectrophotometer analysis, respectively. For first-strand cDNA synthesis, 1 μg of high-quality total RNA was used for reverse transcription with a ReverTra Ace-R kit (Toyobo Co., Ltd., Osaka, Japan). A 20-fold dilution of the resulting cDNA was used as a template for quantitative real-time PCR.

Quantitative Real-Time PCR. The sequences of FtPSY, FtPDS, FtZDS, FtLCYB, FtLCYE, FtCHXB, FtCHXE, FtZEP, FtCCD1, and FtNCED (accession no. KC571227, KC571228, KC571229, KC571230, KC571231, KC571232, KC571233, KC571234,

Table 1. Primers Used for qRT-PCR

gene	forward (5′ to 3′)	reverse (5′ to 3′)
FtPSY	AAATGCCTCACACATAACACCAACT	TTCTTAGGTCCATTCTCATCCCTTC
FtPDS	ACTTGGTGGTGATGTGCAACTTAAT	GGGATTTCTTTCCAGTCTTCAGGTA
FtZDS	CAAAAGGTTGTTACCACAAGAATGG	GTTGTCTAACCCAGTTGCTTCCTTT
FtLCYB	ATAGAGAGGAGAAGGCAAAGGGAGT	GAACAACCTAGACGACAAGAATCCA
FtLCYE	CTAGGGTTTCTGTGCAAACTGCTTA	ACTCAGGGGTCTGAACATCATTTTT
FtCHXB	GAGAAACTGGCTAGGAAGGAATCTG	TGAAATCGGTAGTAAACAGCCAAGA
FtCHXE	AGACAAATAAAAGCCGAAAAAGCTG	CTGCCTCGTTGACATATTCTTCACT
FtZEP	AACTTGCTTTGGAACTGGATAAAGC	AACGATAATGGACCAAGTCCTTCAC
FtCCD1	GAAGTCGGAGGAAATGTTAAAGGAA	TTCTTGCATCAATGACGTAGACAAA
FtNCED	TTAAGTGGATTGATGTCCCTGATTG	TCGTCGCATTCATTAAAAACAGAGT
FtActin	GAGTTATGAGCTTCCTGATGGACAA	CCGCCACTCAACACAATGTTATTAT

KC571235, and KC571236) were used to design real-time PCR primers with the Primer3 Web site (http://frodo.wi.mit.edu/primer3/; Table 1). The expression of these genes was determined using the method of relative quantification with the *F. tataricum* actin housekeeping gene (KC571237) as a reference. Real-time PCR analyses were conducted in a 20 μL reaction mix that contained 5 μL of template cDNA, 10 μL of 1× SYBR Green Real-time PCR Master Mix (Toyobo Co., Ltd., Osaka, Japan), 0.5 μL of each primer (10 μM), and diethylpyrocarbonate water. Thermal cycling conditions were as follows: 95 °C for 5 min and 40 cycles of 95 °C for 15 s, 56 °C for 15 s, and 72 °C for 20 s. PCR products were analyzed using Bio-Rad CFX Manager 2.0 software. Three replications for each sample were used for the real-time analysis.

Carotenoid Extraction and Analysis. Carotenoids were released from the sprout samples (0.02 g) by adding 3 mL of ethanol containing 0.1% ascorbic acid (w/v), vortex mixed for 20 s, and incubated for 5 min in a water bath at 85 °C. The carotenoid extract was saponified using potassium hydroxide (120 μL, 80% w/v) in the 85 °C water bath for 10 min. After saponification, the samples were immediately placed on ice, and cold deionized water (1.5 mL) was added. β-Apo-8′-carotenal (0.2 mL, 25 g/mL) was added as an internal standard. Carotenoids were extracted twice with hexane (1.5 mL) by centrifugation at 1200*g* to separate the layers. Aliquots of the extracts were dried under a stream of nitrogen and redissolved in 50:50 (v/v) dichloromethane/methanol before analysis by using HPLC. The carotenoids were separated on a C30 YMC column (250 × 4.6 mm, 3 μm; Waters Corp., Milford, MA, USA) by using Agilent 1100 HPLC (Agilent Technologies France, Massy, France) that was equipped with a photodiode array detector. Chromatograms were generated at 450 nm. Solvent A consisted of methanol/water (92:8 v/v) with 10 mM ammonium acetate. Solvent B consisted of 100% methyl *tert*-butyl ether. Gradient elution was performed at 1 mL/min under the following conditions: 0 min, 90% A/10% B; 20 min, 83% A/17% B; 29 min, 75% A/25% B; 35 min, 30% A/70% B; 40 min, 30% A/70% B; 42 min, 25% A/75% B; 45 min, 90% A/10% B; and 55 min, 90% A/10% B. Carotenoid standards were purchased from CaroteNature GmbH (Lupsingen, Switzerland). For quantification, calibration curves were drawn by plotting four different concentrations of carotenoid standards according to the peak area ratios by using β-apo-8′-carotenal.

RESULTS AND DISCUSSION

Development of Tartary Buckwheat Sprouts Exposed to White, Blue, and Red LEDs. The development of tartary buckwheat sprouts under different wavelengths of lights for 10 DAS is shown in Figure 2. The sprouts exposed to red light showed the highest lengths and fresh weights, whereas those exposed to blue light were the smallest in lengths and fresh weights (data not shown). The hypocotyls of sprouts irradiated with blue light were dark red, whereas those of sprouts irradiated with white and red light were pale red and white, respectively.

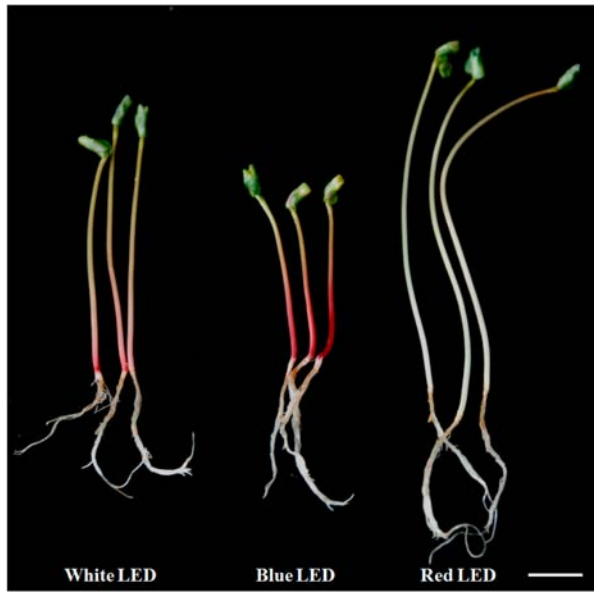

Figure 2. Photograph of tartary buckwheat sprouts exposed to white, blue, and red LEDs at 2 days after sowing. The scale bars represent 1 cm.

Expression Levels of Carotenoid Biosynthetic Genes during Sprout Development of Tartary Buckwheat under White, Blue, and Red LEDs. Tartary buckwheat sprouts were irradiated with white, blue, and red LEDs for 10 days, and changes in the expression levels of carotenoid biosynthetic genes were determined every 2 days by using quantitative real-time PCR (Figure 3). Under white light, the expression patterns of genes related to the carotenoid biosynthetic pathway were essentially similar. Specifically, the expression levels of *FtPSY*, *FtPDS*, *FtZDS*, *FtLYCB*, *FtLCYE*, *FtCHXB*, *FtCHXE*, *FtZEP*, and *FtNCED* were increased from 2 DAS, reached the highest levels at 8 DAS, and then remarkably decreased at 10 DAS. Similar to the results of white light exposure, blue light exposure caused increased transcript levels of genes related to the carotenoid biosynthetic pathway after 2 DAS, which peaked at midstage of the cultivation and finally decreased at 10 DAS. This was also the expression pattern of *FtPSY*, *FtPDS*, *FtZDS*, *FtLYCB*, *FtCHXE*, *FtZEP*, *FtCCD1*, and *FtNCED* in tartary buckwheat sprouts grown under red light during the 10 days of cultivation. The mRNA levels of *FtLCYE* and *FtCHXB* were reduced from 2 to 10 DAS under red light during sprout development. There were no vast differences in the expression levels of carotenoid biosynthetic genes at 2, 4, 6,

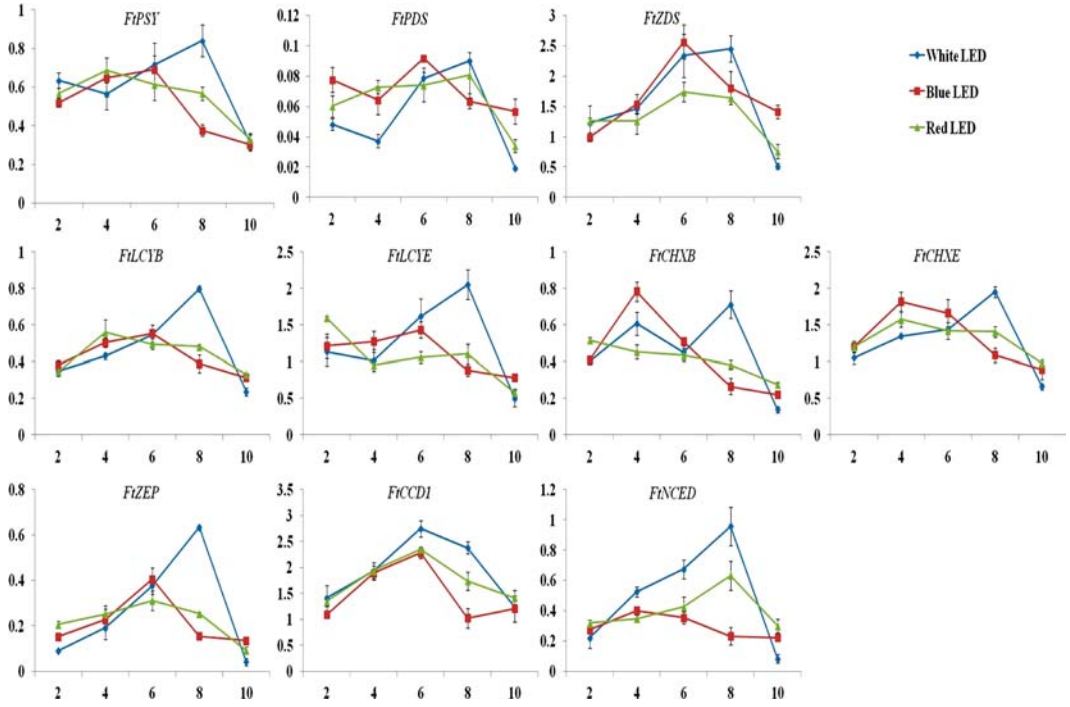

Figure 3. Expression levels of carotenoid biosynthetic genes in tartary buckwheat sprouts exposed to white, blue, and red LEDs. The height of each bar and the error bars indicate the means and standard errors, respectively, from three independent measurements. The vertical axes show the expression relative to *Actin*. Units on the horizontal axes indicate the days after sowing.

Table 2. Carotenoid Composition and Contents in Tartary Buckwheat Sprouts Exposed to White, Blue, and Red Light-Emitting Diodes[a]

DAS	α-carotene	lutein	β-carotene	9-*cis*-β-carotene	13-*cis*-β-carotene	β-cryptoxanthin	zeaxanthin	total carotenoids
				White LED				
2	1.34 ± 0.52	365.29 ± 15.30	187.42 ± 15.04	16.93 ± 1.33	20.65 ± 2.26	0.82 ± 0.05	0.74 ± 0.06	593.19 ± 34.57
4	1.44 ± 0.11	565.25 ± 36.76	297.59 ± 25.58	24.73 ± 2.44	28.61 ± 2.28	0.84 ± 0.12	1.09 ± 0.04	919.55 ± 67.32
6	1.76 ± 0.10	659.15 ± 34.81	369.66 ± 23.58	30.90 ± 2.69	35.71 ± 3.89	0.63 ± 0.08	1.77 ± 0.12	1099.59 ± 65.26
8	1.82 ± 0.11	666.46 ± 11.63	393.71 ± 23.60	32.79 ± 2.46	36.59 ± 4.28	0.55 ± 0.03	1.70 ± 0.06	1133.63 ± 42.17
10	2.07 ± 0.13	735.54 ± 62.68	462.74 ± 34.53	37.21 ± 2.35	41.51 ± 9.72	0.59 ± 0.03	2.96 ± 0.23	1282.63 ± 109.66
				Blue LED				
2	0.92 ± 0.04	343.97 ± 38.31	158.69 ± 10.02	15.89 ± 0.82	17.24 ± 1.39	1.17 ± 0.06	1.45 ± 0.18	539.33 ± 50.82
4	1.30 ± 0.08	424.44 ± 29.41	250.42 ± 9.27	22.16 ± 3.12	24.07 ± 2.10	1.04 ± 0.10	1.83 ± 0.18	725.28 ± 44.26
6	1.58 ± 0.34	512.82 ± 37.75	322.60 ± 15.01	26.87 ± 5.63	29.86 ± 3.98	0.80 ± 0.15	3.08 ± 0.56	897.61 ± 63.42
8	1.63 ± 0.12	544.53 ± 42.87	331.22 ± 18.94	28.53 ± 1.89	30.64 ± 2.99	0.60 ± 0.15	3.71 ± 0.19	940.86 ± 67.15
10	1.37 ± 0.02	509.56 ± 42.06	289.98 ± 12.17	24.65 ± 0.40	27.41 ± 2.74	0.57 ± 0.15	4.76 ± 0.46	858.29 ± 58.00
				Red LED				
2	2.17 ± 0.46	415.43 ± 40.41	217.70 ± 9.71	19.61 ± 0.39	21.93 ± 4.65	0.86 ± 0.06	1.44 ± 0.13	679.13 ± 55.81
4	1.41 ± 0.16	574.08 ± 51.37	296.66 ± 27.01	24.44 ± 3.18	29.49 ± 2.34	0.66 ± 0.13	2.49 ± 0.25	929.21 ± 84.45
6	1.55 ± 0.18	606.80 ± 51.21	315.57 ± 30.43	27.44 ± 3.61	30.22 ± 2.67	0.45 ± 0.07	3.51 ± 0.32	985.54 ± 88.48
8	1.50 ± 0.07	565.95 ± 41.16	317.37 ± 19.96	26.59 ± 1.76	30.06 ± 2.74	0.49 ± 0.07	3.69 ± 0.32	945.65 ± 66.09
10	1.35 ± 0.14	544.28 ± 18.19	305.28 ± 27.72	24.02 ± 2.55	28.64 ± 0.13	0.46 ± 0.16	4.62 ± 0.27	908.64 ± 49.16

[a]The results are expressed as means ± standard errors from three independent measurements. Values are expressed as $\mu g\ g^{-1}$ dry weight. DAS, days after sowing.

and 10 DAS under the three different light treatments; however, the expression levels of *FtPSY*, *FtLCYB*, *FtLCYE*, *FtCHXB*, *FtCHXE*, and *FtZEP* were remarkably higher at 8 DAS in sprouts grown under white light than in those grown under blue and red lights.

Analysis of Carotenoid Content during Sprout Development of Tartary Buckwheat under White, Blue, and Red LEDs. The same plant materials as those used for quantitative real-time PCR were used to analyze the composition and content of carotenoids in the sprouts of tartary buckwheat by using HPLC (Table 2). In general, the dominant carotenoids in tartary buckwheat sprouts were lutein and β-carotene. Small amounts of 9-*cis* β-carotene and 13-*cis* β-carotene were also detected, whereas only trace amounts of α-carotene, β-cryptoxanthin, and zeaxanthin were found in tartary buckwheat sprouts. Under white light, the accumulation of lutein and β-carotene was induced along with increases in the total carotenoid content after sowing, which reached the highest content at 10 DAS. The accumulation patterns of carotenoids in sprouts under blue and red lights were generally

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similar; they increased from 2 to 6 DAS (red light) or 8 DAS (blue light) and then decreased at 10 DAS. The amounts of carotenoids in sprouts exposed to white light were relatively higher than those in sprouts treated with blue and red lights (Table 2). The maximum production of total carotenoids of tartary buckwheat sprouts under white, blue, and red lights was 1282.63 μg g^{-1} dry weight (DW) (10 DAS), 940.86 μg g^{-1} DW (8 DAS), and 985.54 μg g^{-1} DW (6 DAS), respectively.

Carotenoids play an important role during photosynthesis in chloroplasts from green organs. Therefore, carotenoid biosynthesis is influenced by light quality, intensity, and duration, and not all wavelengths of light are required for equal efficiency.[26,28] In tartary buckwheat sprouts, the expression levels of genes related to carotenoid biosynthesis did not show substantial differences under white, blue, and red lights from 2 to 6 DAS. However, the transcription levels of *FtPSY*, *FtLCYB*, *FtLCYE*, *FtCHXB*, *FtCHXE*, and *FtZEP* were higher in sprouts grown under white light than in those grown under blue and red lights. The expression of these mRNAs might lead to the rich amounts of carotenoids during the late stage of cultivation in sprouts exposed to white light. The abundant accumulation of lutein and β-carotene was probably responsible for the low amounts of their precursors and derivatives in the ε,β-carotenoid and β,β-carotenoid branches, respectively.

At present, sprouts have received widespread attention as natural functional foods because of their high nutritive values and protein, mineral, and vitamin contents compared to those found in raw seeds, as well as because of their simple and inexpensive production.[29] In addition, the demand for achieving high intake of carotenoids without using genetically modified plants is particularly high. In this study, we found that tartary buckwheat sprouts exposed to white LED at 10 DAS contained very high amounts of total carotenoids (1282.63 μg g^{-1}), which was higher than those found in sprouts grown under blue (940.86 μg g^{-1}) and red (985.54 μg g^{-1}) LEDs. These results are different from previous studies, which found that the accumulation of carotenoids in the flavedo of citrus fruits exposed to red LED and citrus sacs in vitro treated with blue LED were significantly higher than that of citrus fruits and sacs exposed to other lights.[25,30] The data indicate that the effects of different wavelengths of light on carotenoid biosynthesis may be dependent on the tissues and plants examined. In addition, the total carotenoid amount of sprouts exposed to white LED was considerably higher than that of sprouts grown under standard cool white fluorescent tubes with a flux rate of 35 μmol s^{-1} m^{-2} (573.96 μg g^{-1} DW), as reported in our previous study.[9] Therefore, this study might establish an effective strategy for improving the carotenoid production of the tartary buckwheat sprouts. Because LED is commercially available and can be readily and easily applied to sprout cultures, it might be useful for the large-scale production of nutritional tartary buckwheat sprouts in the future.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

DEPC, diethylpyrocarbonate; DAS, days after sowing; HPLC, high-performance liquid chromatography; LED, light-emitting diode; GGDP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ξ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CHXB, β-ring carotene hydroxylase; CHXE, ε-ring carotene hydroxylase; ZEP, zeaxanthin epoxidase; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase

REFERENCES

(1) Fabjan, N.; Rode, J.; Košir, I. J.; Wang, Z. H.; Zhang, Z.; Kreft, I. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. *J. Agric. Food Chem.* **2003**, *51*, 6452−6455.

(2) Guo, X. D.; Ma, Y. J.; Parry, J.; Gao, J. M.; Yu, L. L.; Wang, M. Phenolics content and antioxidant activity of tartary buckwheat from different locations. *Molecules* **2011**, *16*, 9850−9867.

(3) Bonafaccia, G.; Marocchini, M.; Kreft, I. Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chem.* **2003**, *80*, 9−15.

(4) Suzuki, T.; Honda, Y.; Mukasa, Y. Effects of UV-B radiation, cold and desiccation stress on rutin concentration and rutin glucosidase activity in tartary buckwheat (*Fagopyrum tataricum*) leaves. *Plant Sci.* **2005**, *168*, 1303−1307.

(5) Ren, Q.; Wu, C.; Zhang, J. Use of on-line stop-flow heart-cutting two-dimensional high performance liquid chromatography for simultaneous determination of 12 major constituents in tartary buckwheat (*Fagopyrum tataricum* Gaertn). *J. Chromatogr., A* **2013**, *1304*, 257−262.

(6) Liu, C. L.; Chen, Y. S.; Yang, J. H.; Chiang, B. H. Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. *J. Agric. Food Chem.* **2008**, *56*, 173−178.

(7) Yao, Y.; Shan, F.; Bian, J.; Chen, F.; Wang, M.; Ren, G. D-Chiro-inositol-enriched tartary buckwheat bran extract lowers the blood glucose level in KK-Ay mice. *J. Agric. Food Chem.* **2008**, *56*, 10027−10031.

(8) Kim, S. J.; Zaidul, I. S. M.; Suzuki, T.; Mukasa, Y.; Hashimoto, N.; Takigawa, S.; Noda, T.; Matsuura-Endo, C.; Yamauchi, H. Comparison of phenolic compositions between common and tartary buckwheat (*Fagopyrum*) sprouts. *Food Chem.* **2008**, *110*, 814−820.

(9) Tuan, P. A.; Thwe, A. A.; Kim, J. K.; Kim, Y. B.; Lee, S.; Park, S. U. Molecular characterisation and the light−dark regulation of carotenoid biosynthesis in sprouts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.). *Food Chem.* **2013**, *141*, 3803−3812.

(10) Britton, G. Overview on carotenoid biosynthesis. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser: Basel, Switzerland, 1998; Vol. 3, pp 13−147.

(11) Howitt, C. A.; Pogson, B. J. Carotenoid accumulation and function in seeds and non-green tissues. *Plant Cell Environ.* **2006**, *29*, 435−445.

(12) Frank, H. A.; Cogdell, R. J. Carotenoids in photosynthesis. *Photochem. Photobiol.* **1996**, *63*, 257−264.

(13) Ledford, H. K.; Niyogi, K. K. Singlet oxygen and photooxidative stress management in plants and algae. *Plant Cell Environ.* **2005**, *28*, 1037−1045.

(14) Havaux, M. Carotenoids as membrane stabilizers in chloroplasts. *Trends Plant Sci.* **1998**, *3*, 147−151.

(15) Mactier, H.; Weaver, L. T. Vitamin A and preterm infants: what we know, what we don't know and what we need to know. *Arch. Dis. Child.* **2005**, *90*, 103−108.

(16) Villamor, E.; Fawzi, W. W. Vitamin A supplementation: implications for morbidity and mortality in children. *J. Infect. Dis.* **2000**, *182*, S122−S133.
(17) Thorne-Lyman, A. L.; Fawzi, W. W. Vitamin A and carotenoids during pregnancy and maternal, neonatal and infant health outcomes: a systematic review and meta-analysis. *Paediatr. Perinat. Epidemiol.* **2012**, *26* (Suppl. 1), 36−54.
(18) Mayne, S. T. β-Carotene, carotenoids, and disease prevention in humans. *FASEB J.* **1996**, *10*, 690−701.
(19) Giovannucci, E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J. Natl. Cancer Inst.* **1999**, *91*, 317−331.
(20) Ziegler, R. G. Vegetables, fruits, and carotenoids and the risk of cancer. *Am. J. Clin. Nutr.* **1991**, *53*, 251S−259S.
(21) Gaziano, J. M.; Hennekens, C. H. The role of β-carotene in the prevention of cardiovascular disease. *Ann. N.Y. Acad. Sci.* **1993**, *691*, 148−155.
(22) Cunningham, F. X.; Gantt, E. Genes and enzymes of carotenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1998**, *49*, 557−583.
(23) Hannoufa, A.; Hossain, Z. Regulation of carotenoid accumulation in plants. *Biocatal. Agric. Biotechnol.* **2012**, *1*, 198−202.
(24) Ye, X.; Al-Babili, S.; Klöti, A.; Zhang, J.; Lucca, P.; Beyer, P.; Potrykus, I. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **2000**, *287*, 303−305.
(25) Ma, G.; Zhang, L.; Kato, M.; Yamawaki, K.; Kiriiwa, Y.; Yahata, M.; Ikoma, Y.; Matsumoto, H. Effect of blue and red LED light irradiation on β-cryptoxanthin accumulation in the flavedo of citrus fruits. *J. Agric. Food Chem.* **2012**, *60*, 197−201.
(26) Wu, M. C.; Hou, C. Y.; Jiang, C. M.; Wang, Y. T.; Wang, C. Y.; Chen, H. H.; Chang, H. M. A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. *Food Chem.* **2007**, *101*, 1753−1758.
(27) Lefsrud, M. G.; Kopsell, D. A.; Sams, C. E. Irradiance from distinct wavelength light-emitting diodes affect secondary metabolites in kale. *HortScience* **2008**, *43*, 2243−2244.
(28) Liu, J. H. Regeneration and characterization of plants derived from leaf in vitro culture of two sweet orange (*Citrus sinensis* (L.) Osbeck) cultivars. *Sci. Hortic.* **2009**, *120*, 70−76.
(29) Waje, C. K.; Jun, S. Y.; Lee, Y. K.; Kim, B. N.; Han, D. H.; Jo, C.; Kwon, J. H. Microbial quality assessment and pathogen inactivation by electron beam and gamma irradiation of commercial seed sprouts. *Food Control* **2009**, *20*, 200−204.
(30) Zhang, L.; Ma, G.; Kato, M.; Yamawaki, K.; Takagi, T.; Kiriiwa, Y.; Ikoma, Y.; Matsumoto, H.; Yoshioka, T.; Nesumi, H. Regulation of carotenoid accumulation and the expression of carotenoid metabolic genes in citrus juice sacs in vitro. *J. Exp. Bot.* **2012**, *63*, 871−886.