Enhancement of accumulation of bioactive compounds in red leaf lettuce by manipulation of UV light before harvest

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Abstract

In a plant factory with artificial light, accumulation of bioactive compounds can be enhanced via precise environmental control. Light is essential for growth and development, and for the production of bioactive compounds in plants. Ultraviolet (UV) light stimulates bioactive compound biosynthesis in plants including antioxidants. We developed a new and economical UV-rich fluorescent lamp (UV-FL), with the ability to adjust the ratio of UV-A and UV-B. The objective of this study was to determine the effect of UV light on the accumulation of bioactive compounds in red leaf lettuce. Red leaf lettuce plants were grown in a plant factory with normal growing conditions under white light as light source (23/20°C, 70% RH, 16 h light period, PPFD 150 µmol m^{-2} s^{-1}, 1,000 ppm CO_2) for 23 days. The plants were subjected to UV light under white light (low, middle, and high; 0.3, 0.6, and 0.9 W m^{-2}) for 3 days before harvest. Fresh and dry weights of shoot, leaf area, total chlorophyll, total phenolic concentration, and ORAC for antioxidant activity were measured before and after the UV treatments. Middle and high UV treatments resulted in negative effects on the growth characteristics such as shoot fresh and dry weight, and leaf area. However, low UV treatment was not significantly different from the control for shoot dry weight. Total chlorophyll was higher in low and middle UV treatments than the high UV treatment and the control. In contrast, total phenolic concentration and ORAC value of lettuce plants grown under UV treatments were significantly higher than that of the control. This study suggests that short-term UV irradiation using economical UV-FL lamp before harvest was effective in improving the vegetable quality.

Keywords: abiotic stress, antioxidant capacity, phenolics, plant factory, phytochemical

INTRODUCTION

Among the closed-type plant production systems, a plant factory with artificial light is an agricultural system that can produce crops in an industrially stable manner by controlling the environmental conditions such as light, temperature, nutrient, and moisture within facilities regardless of the external weather. It is possible to improve bioactive compounds accumulation in plants through precise control of the environment (Kozai, 2013). Large-scale plant factory under controlled environmental conditions is a major boon for the plant industry because it enables rapid and mass production of crops with optimal quality (Goto, 2003). In closed-type plant production systems, additional costs are incurred on artificial light instead of sunlight. This alone necessitates higher yields and better crop quality to ensure crop cultivation is economically feasible (Piovene et al., 2015). Therefore, a strategy for improving phytochemicals of crops is an approach to enhance high-value crops in a plant factory (Um et al., 2010).

Environmental stress generates reactive oxygen species (ROS) as a defense mechanism in plants. Plants accumulate more secondary metabolites than primary metabolites to scavenge ROS produced by environmental stress conditions (Behn et al., 2011). Accumulation of antioxidants in plants are one of the defense mechanisms used to overcome stress

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(Blokhina et al., 2003). Therefore, stress has been used to improve health-promoting phytochemicals in plants (Kirakosyan et al., 2004). These findings suggest ways to improve the quality of fruits and vegetables using environmental stress conditions (Oh et al., 2009).

Among the many environmental factors, light is one of the essential energy sources and a key factor to produce bioactive compounds as well as plant growth and development (Smith, 1982). The strong ultraviolet (UV; 280-400 nm) radiation damages DNA, RNA, proteins, chloroplast, and photosynthetic pigments in plants and causes generation of ROS (Hideg et al., 2013). Accordingly, UV radiation generally stresses plants and affects growth and development (Lidon et al., 2012) in various ways, for instance, damaging DNA molecules (Buma et al., 2001), inhibiting photosynthesis (Han et al., 2003), and affecting enzymatic activities (Dring et al., 2002). Meanwhile, ROS produced in response to UV rays may act as signaling molecules (Pitzschke et al., 2006) and generate antioxidants by activating gene expression or the stress defense mechanism in plants (Hideg et al., 2013; A.-H.-Mackerness et al., 2001). In this respect, UV irradiation may be an effective elicitor that can stimulate the biosynthesis of bioactive compounds in cultivated plants.

The primary objective of this study was to examine the effect of the commercially-developed fluorescent lamps with a UV spectrum (UV-FL) on the accumulation of bioactive compounds in red leaf lettuce.

MATERIALS AND METHODS

Plant materials and UV treatment

One-day-old germinated red leaf lettuce seeds (*Lactuca sativa* 'Red Fire', Takii seedling Co., Ltd.) were sown in urethane foam cubes and maintained at normal growing conditions (23/20°C temperature, 70% RH, 16 h light period, PPFD 150 μmol m^{-2} s^{-1} provided by white fluorescent lamp (FHF 32-EX-N-H, Panasonic Corp.), 1,000 ppm CO_2) for seedlings in a plant factory. Sixteen days after sowing (DAS), each lettuce seedling was transplanted and cultivated in a DFT hydroponic system for 7 days before UV treatment.

For UV treatment, a newly developed economical UV-rich fluorescent lamp (UV-FL) (Panasonic Corp.) was used with white LEDs: (1) W LEDs (control), (2) W LEDs with low level of UV-FL (UV low), (3) W LEDs with mid-level of UV-FL (UV mid), and (4) W LEDs with high levels of UV-FL (UV high). The difference in PPFD for each treatment was about 150 μmol m^{-2} s^{-1} between the treatments (Figure 1). Twenty-three DAS the lettuce plants were subjected to each treatment for 3 days.

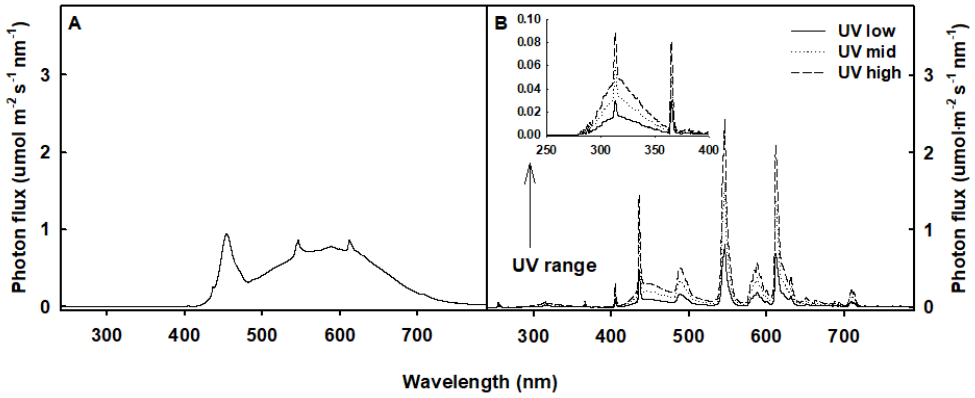

Figure 1. Spectral distribution of white LEDs (A; control) and UV-FL (B) used in this study and measured using a spectro-radiometer (USR-45DA; USHIO Inc.). UV low, 0.3 W m^{-2}; UV mid, 0.6 W m^{-2}; UV high, 0.9 W m^{-2}. The intensity of UV-FL can be adjusted manually by the controller.

Measurement parameters

To determine the growth characteristics, the fresh and dry weights of shoot, total leaf area, specific leaf weight (SLW), and chlorophyll (Chl) content were measured after treatment of plans with UV radiation. The SLW was calculated by dividing the shoot dry weight (mg) by the leaf area. Total Chl content was determined as described in Son and Oh (2015) and expressed as μg Chl per gram (g) fresh weight of lettuce leaves. To determine the total phenolic concentration and antioxidant capacity, approximately 0.1, and 0.05 g of each ground sample was extracted in 3 mL of 80% acetone and 1 mL of 75 mmol L^{-1} phosphate buffer (pH 7.4) using an automatic grinder (Mixer Mill MM 200; Retch, Haan, Germany) operating at 30 Hz for 6 min. The homogenate was centrifuged at 20,500×g at 4°C for 15 min. Total phenolic content was determined using the Folin-Ciocalteu reagent method (Ainsworth and Grillespie, 2007) and expressed as the gallic acid-equivalent (GAE; mg). Antioxidant capacity was determined using oxygen radical capacity (ORAC) assay following a modified method of Zhao et al. (2007) and expressed as μmol Trolox equivalents per gram (g) shoot fresh mass (mg GAE g^{-1} FW, and mM Trolox equivalents g^{-1} FW).

Statistical analysis

All parameters were measured using eight plants per treatment. Data were analyzed using the statistical software program (SAS 9.2; SAS Institute, Cary, NC, USA). One-way ANOVA was performed using SAS, and Duncan's multiple range test was used for analyzing significant differences between means.

RESULTS AND DISCUSSION

Growth characteristics

Lettuce plants exposed to UV light had significantly decreased shoot fresh and dry weights at 3 days after UV treatment compared with that of the control (Table 1). However, shoot dry weight of lettuce plants exposed to UV-low treatment had no significant difference when compared with that of the control. Leaf area also had similar trends as with the shoot fresh weight. Total Chl content of lettuce plants exposed to UV treatments were not significantly decreased compared with that of the control.

Table 1. Growth characteristics of lettuce plants subjected to various UV-FL for 3 days. Control, white LEDs; UV low, 0.3 W m^{-2}; UV mid, 0.6 W m^{-2}; UV high, 0.9 W m^{-2} (n=8).

Treatment	Shoot weight (g)		Leaf area (cm^2)	SLW (mg cm^{-2})	Total chlorophyll (μg g^{-1} FW)
	Fresh	Dry			
Control	7.60 a	0.39 a	201.42 a	1.86	189.45 b
UV low	5.91 b	0.36 a	138.12 b	2.42	182.50 b
UV mid	3.46 c	0.23 b	83.40 c	2.55	212.35 a
UV high	4.26 c	0.28 b	99.03 c	2.63	214.48 a

Different letters within columns indicate significant differences (P=0.05) by Duncan's multiple range test.

As solar UV range is generally regarded as a negative environmental factor in higher plants, negative effects of the UV wavelength on plant growth and development have been widely reported. Such negative effects on plant growth are known to result from damage to D1 protein in PS II due to UV irradiation (Vass, 1997). In this study, inhibition of lettuce growth when subjected to UV treatments could be attributed to a decrease in PS II activation. Lee et al. (2014) observed that the inhibition of growth in lettuce plants was more pronounced in the UV-C lamp than those with UV-A or -B lamps. Goto et al. (2016) reported that lettuce plants exposed to UV-A and B LEDs had no signs of growth impediment. Thus, UV-FL used in this study, containing the UV-C (200-280 nm) radiation might have inhibited the growth of lettuce plants rather than UV-B. Reports showed that UV-A (315-380 nm) radiation had a positive effect on chlorophyll and carotenoids (Helsper et al., 2003). Štroch et al. (2015) found that barley plants subjected to UV-A had higher Chl content than those not exposed to UV-A. They

described that the UV-A region could be absorbed by chlorophylls. This result is consistent with our finding that Chl content under UV treatments showed no significant differences compared with that of the control. Thus, it implied that the UV-FL used in this study, containing the UV-A radiation, could be used without inhibiting the Chl synthesis.

Total phenolic concentration and antioxidant capacity

All UV treatments significantly induced the accumulation of phenolic compounds and antioxidant capacity at 3 days after the UV treatment (Figure 2). Lettuce plants exposed to a high level of UV treatment showed the maximum phenolic and antioxidant levels between the treatments.

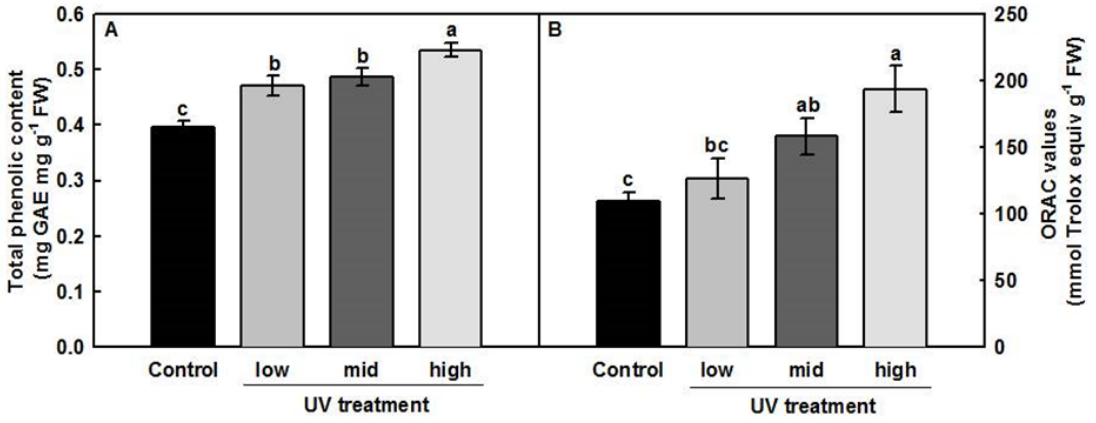

Figure 2. Total phenolic content (A) and antioxidant capacity (ORAC) (B) of lettuce plants subjected to various UV-FL for 3 days. Control, white LEDs; UV low, 0.3 W m^{-2}; UV mid, 0.6 W m^{-2}; UV high, 0.9 W m^{-2}. The data shown are the means and the vertical bars indicate standard errors (n=8). Significant at P=0.01.

The strong energy of UV light led to the generation of ROS capable of damaging DNA, RNA, proteins, chloroplasts, and photosynthetic pigments in plants (Hideg et al., 2013). UV-absorbing compounds such as phenolics and flavonoids were accumulated due to UV irradiation as it is one of the defense mechanisms used by plants (Jenkins, 2009). This is consistent with our result in that the total phenolic content was enhanced by UV irradiation. This result may be explained by the generation of ROS due to exposure to excessive UV energy. UV-A wavelength induces antioxidant properties in plants as a defense mechanism against environmental stresses (Lee et al., 2014). They also found increased levels of total phenolics and antioxidant capacity in red-leaf lettuce plants subjected to UV-B or -C lamp. UV-B light stimulated the biosynthesis of anthocyanin and other antioxidant polyphenols in lettuce (Goto et al., 2016). UV irradiation typically accompanied the accumulation of polyphenolics including phenolic acids and flavonoids in plants (Jansen et al., 1998). This may be explained by the biosynthesis of the phenolics and antioxidants to protect the plants from damages caused by exposure to UV. Therefore, UV-FL used in this study, containing various ranges of UV-A, B, and C, may have stimulated the synthesis of diverse phytochemicals in lettuce plant.

CONCLUSIONS

This study suggested that the application of UV may be useful to improve lettuce quality, especially phenolic compounds and antioxidant properties in plants before harvest. UV-FL emits UV radiation of various wavelengths, and therefore, the use of UV-FL might improve crop quality in a closed-type plant factory. However, there is limited information about a specific wavelength of UV-C on plant growth and phytochemicals. Most studies that have used UV lamps did not describe the spectral distribution (composition) nor the effects of UV-C, even though UV lamps included the UV-C wavelength. Therefore, further research is required to

explore the effects of UV-C or describe the effect of specific UV wavelength in plants.

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