Supplementary Ultraviolet Radiation B Together with Blue Light at Night Increased Quercetin Content and Flavonol Synthase Gene Expression in Leaf Lettuce (*Lactuca sativa* L.)

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Establishment of an effective supplementary lighting procedure is necessary to increase the value of leaf lettuce grown using a hydroponic method involving a low production cost. In leaf lettuce extracts, quercetin, one of the flavonoids, was isolated and identified. It was investigated that quercetin has important functions that can be used as a dietary supplement. Flavonol synthase (FLS) is a key enzyme involved in quercetin biosynthesis, catalyzes the conversion of dihydroquercetin to quercetin. Therefore, we determined the sequence of the flavonol synthase gene (FLS) in red leaf lettuce. We harvested leaf lettuce grown using supplementary light sources, such as ultraviolet radiation B (UV-B), ultraviolet radiation A, blue, and red lamps during the night. It is noteworthy that FLS expression and the quercetin content were particularly increased to a greater extent in young leaves than in mature leaves when UV-B and blue light were used simultaneously at night. We suggest that UV-B with blue light is used simultaneously at night for producing leaf lettuce with high quercetin content.

Keywords: blue light, flavonol synthase gene, leaf lettuce, quercetin, ultraviolet radiation B

INTRODUCTION

Although the hydroponic cultivation of leaf lettuce (*Lactuca sativa* L.) in greenhouses and plant factories has expanded rapidly, it is reported that the flavonoid content of leaf lettuce grown in such environments decreases compared with that of leaf lettuce grown under field conditions (Shoji et al., 2001; Voipio and Autio 1995). In recent years, consumer interest has been increasing in functional foods containing flavonoids (Maeda et al., 2006). Therefore cultivation techniques are required that increase the value of vegetables (Goto and Takakura, 2003; Goto, 2003) and de-

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Abbreviations: cDNA, complementary DNA; CHI, chalcone isomerase; CHS, chalcone synthase; EC, electrical conductivity; FLS, flavonol synthase; F3H, flavonoid 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; FW, flesh weight; HPLC, high-performance liquid chromatography; PPFD, photosynthetic photon flux density; qPCR, quantitative real-time reverse-transcription polymerase chain reaction; RT-PCR, reverse-transcription polymerase chain reaction; UV-A, ultraviolet radiation A; UV-B, ultraviolet radiation B.
crease the production cost (Shimizu et al., 2004).

The structures of flavonoids from many plants have been documented (Dixon et al., 2004; Williams and Grayer, 2004; Knaggs, 2001). Flavonoids play important roles in the value of plant-derived foods, because they affect characteristics such as appearance, flavor, and health-promoting properties (Finley, 2005; Hubbard et al., 2004 and 2003; Tomas-Barberan and Espin, 2001; Middleton et al., 2000). Flavonols (classified as flavonoids) can exhibit free radical scavenging and antioxidant activities (Moreira et al., 2005; Lugasi et al., 2003; Choi et al., 2003; Middleton et al., 2000) and can be important as protective agents against damage caused by UV irradiation (Kaffarnik et al., 2005; Turunen et al., 1999; Middleton and Teramura, 1993). It is important that flavonol content increases in vegetables.

The combined results of genetic, biochemical, and molecular studies have provided a comprehensive understanding of many important biosynthetic pathways of flavonols (Stracke et al., 2007; Routaboul et al., 2006; Tohge et al., 2005; Winkel-Shirley et al., 1995). A generalized scheme of the flavonol biosynthetic pathway in Arabidopsis thaliana leaves is shown in Fig. 1. Flavonols are formed at a metabolic branch point in the flavonoid metabolic pathway (Knaggs, 2001; Holton and Cornish, 1995). In A. thaliana, flavonol synthase (FLS) catalyzes the conversion of dihydroquercetin and dihydrokaempferol to quercetin and kaempferol, respectively (Pelletier et al., 1999; Wisman et al., 1998). It is known that quercetin has the effect as antioxidant (Williamson et al., 1996), natural antihistamine (Theoharides and Bielory, 2004), and anti-inflammatory (Donnelly et al., 2004). Quercetin were isolated and identified in leaf lettuce extracts (Ferreres et al., 1997). Therefore, it is considered that FLS expression plays an important role in increasing the quercetin content in the leaf lettuce.

Light quality is one of the most important environmental signals regulating flavonoid biosynthesis (Wade et al., 2001; Jenkins, 1997; Fuglevand et al., 1996). It is confirmed that chalcone synthase (CHS), the key enzyme involved in flavonoid biosynthesis in many plants, is regulated by several environmental and endogenous stimuli, including light (Mackerness, 2000; Chalker-Scott, 1999; Mol et al., 1996). Ultraviolet radiation A (UV-A, 320–400 nm) and blue light (400–500 nm) each act synergistically with ultraviolet radiation B (UV-B, 280–320 nm) to stimulate CHS transcript accumulation in A. thaliana (Wade et al., 2001; Jenkins, 1997; Fuglevand et al., 1996). FLS expression is expected to respond to light in a way similar to the CHS expression (Pelletier et al., 1999; Wisman et al., 1998). In Trifolium repens leaves (Hofmann et al., 2003, 2001, and 2000) and Pinus sylvestris needles (Lavola et al., 2003), UV-B together with white light is important for high flavonol content. There is limited information on the molecular regulation of the flavonoid metabolism and its putative role during flavonol biosynthesis in vegetables (Park et al., 2007).

![Fig. 1](image)

**Fig. 1** The putative flavonol biosynthetic pathway. The reactions in this pathway are catalyzed by chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3′-hydroxylase (F3′H), and flavonol synthase (FLS).
However, it is thought that irradiating different light quality can control quercetin content in leaf lettuce leaves.

In this study, we describe the effects of different supplementary light sources (UV-B, UV-A, blue and red lamps) used at night when the leaf lettuce was cultivated under artificial light in a growth chamber. In red leaf lettuce grown under hydroponic culture, it was reported that the anthocyanin content of young leaves increases to a greater extent than that of mature leaves (Shoji et al., 2001). Therefore, the young and mature leaves of the leaf lettuce were analyzed in this study. We isolated complementary DNA (cDNA) of FLS in red leaf lettuce, analyzed the FLS expression in red leaf lettuce by using quantitative real-time reverse-transcription polymerase chain reaction (qPCR), and determined the quercetin content of three lettuce cultivars by using high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Plant materials and growth conditions

Seeds of red leaf lettuce (Lactuca sativa L. cv. Banchu red fire, Takii seed, Japan), green leaf lettuce (Lactuca sativa L. cv. Green wave, Takii seed, Japan), and butterhead lettuce (Lactuca sativa L. cv. Okayama-saradana, Takii seed, Japan) were sown on urethane cubes in plastic trays filled with water. The trays were covered with aluminum foil and placed in the dark at 25°C for 3 days. The seedlings were grown in a growth chamber (MIR-553, Sanyo, Japan) at 25°C and under a 12 h light period (7:00–19:00) until harvest. When the seedlings were 7-d-old, they were grown hydroponically using Hyponica Fertilizer A and B (Kyowa, Japan). The electrical conductivity (EC) of the hydroponic solution was adjusted to 1.2 dS m⁻¹. When they were 14-d-old, they were transplanted to hydroponic system. The EC of the hydroponic solution was maintained at 2.0 dS m⁻¹ from the second day after the transplanting. The pH of the hydroponic solution was adjusted to 6.5 until harvest. The 28-d-old plants were grown under experimental treatments of different light for 2 weeks, and harvested immediately in the end of the nighttime (6:50–7:00). Samplings were carried out for determination of the FLS expression level in red leaf lettuce and quercetin content in three lettuce cultivars on the same time. The third (young leaves) and the fifth (mature leaves) true leaves for red and green leaf lettuces were collected as analysis samples. The third true leaves developed in the start of the light treatment of the nighttime. The sixth true leaves were too small to analyze quercetin content. In butterhead lettuce, the light treated fourth and eighth true leaves were used for the same reason in the case of red and green leaf lettuce leaves.

Light sources and spectral treatments

The 3-d-old seedlings were cultivated under white light (150 μmol m⁻² s⁻¹) and a 12/12 h (day/night, 7:00–19:00/19:00–7:00) photoperiod. The 14-d-old plants were used for experimental treatments involving exposure to different light for 12 h at night and to white light for 12 h in the daytime. Untreated plants used as a control were cultivated under darkness for 12 h at night and under white light for 12 h in the daytime. In addition, the spectral photon flux, ultraviolet radiation (UV, 280–400 nm) and photosynthetic photon density (PPFD, 400–700 nm) of different fluorescent lamps used for the experiments were measured using a spectroradiometer (MSR7000, Opto Research, Japan), a UV digital radiometer (UV103, Macam Photometrics, UK), and a quantum sensor (LI-190SB, LI-COR, USA), respectively. Fluorescent lamps emitting white light (FLR110HW/ A/100, Toshiba, Japan), UV-B (F20T10UV-B, Sankyo Denki, Japan), UV-A (F20T10BLB, Toshiba, Japan), blue light (FL20T8BC, Elevam, Japan), and red light (FL20T8RK, Elevam, Japan) were suspended at a height of approximately 300 mm above the plant seedlings in the growth chamber. Spectral photon distributions were determined using the spectroradiometer to measure the quality of the different kinds of light used (Fig. 2). Referring to the experiments performed by Shoji et al., (2001) and Wade et al., (2001), the fluence rates of white light (150 μmol m⁻² s⁻¹), UV-
Fig. 2  Spectral photon distributions of fluorescent lamps. Relative spectral photon flux of each lamp was normalized to 1.0 at 544 nm (White), 306 nm (UV-B), 359 nm (UV-A), 454 nm (Blue), and 659 nm (Red).

B (0.5 Wm⁻²), UV-A (9.5 Wm⁻²), blue light (60 μmol m⁻²s⁻¹), and red light (60 μmol m⁻²s⁻¹) were adjusted by changing the height and number of fluorescent lamps.

RNA isolation, gene cloning, and expression analysis

Total RNA was isolated from each sample using an RNeasy Plant Mini Kit (QIAGEN, USA). The isolated RNA was treated with DNase I before cDNA synthesis to avoid contamination of genomic DNA. The extracted total RNA was used as a template for reverse-transcription reaction using the Rever Tra Ace kit (Toyobo, Japan) and oligo dT (20) primer. To clone FLS and actin gene (ACT) from the cDNA fragments, we synthesized the oligonucleotide primer sets (Table 1) based on the conserved regions annotated in the GenBank database. FLS and ACT were partially cloned by reverse-transcription polymerase chain reaction (RT-PCR) using cDNA and primer sets (Table 1) for gene cloning. PCR was performed with 30 cycles of 95°C for 1 min, 55°C for 30 sec and 68°C for 30 sec. The RT-PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen, USA) and transformed into Escherichia coli (TOP10, Invitrogen, USA). The double-stranded plasmid DNA was sequenced using the T7 forward primer, M13 reverse primer, and a BigDye Terminator Cycle sequencing kit (Applied Biosystems, Japan) and identified using BLASTX and the universal protein resource (UniProt) database. The oligonucleotide primer sets were designed based on results of the target-gene (Table 1) to analyze the target-gene expression in the cDNA fragment. The qPCR was performed using SYBR green (Takara-Bio, Japan) on a Smart Cycler (Cepheid, USA) according to the provided manual. The expression levels of the Lactuca sativa flavonol synthase gene (LsFLS) were normalized to those of the Lactuca sativa actin gene (LsACT), which was used as the housekeeping gene.

Extraction and separation of quercetin aglycone

The lettuce leaves grown under various light conditions were harvested and cut into pieces.
Table 1  List of oligonucleotide primers used for gene cloning and expression.

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>For gene cloning</td>
<td>5'-GAGTTCATAAGACCGCAAAACCAAGAAAGGCCCAGCA</td>
<td>5'-TCCAAGAAACTGGCCACGA</td>
</tr>
<tr>
<td>FLS</td>
<td>GGAACAGCCA-3'</td>
<td>CATTCAGTC-3'</td>
</tr>
<tr>
<td>ACT</td>
<td>5'-MGNCAAMHGGHGTBATGACT-3'</td>
<td>5'-GCYTGATRGCAGTACAT-3'</td>
</tr>
<tr>
<td>For gene expression</td>
<td>5'-CCATACAGAATATGCTCTCCAA</td>
<td>5'-GCTCAATATGTCCATTTGGTCA</td>
</tr>
<tr>
<td>LsFLS</td>
<td>TCACC-3'</td>
<td>CC-3'</td>
</tr>
<tr>
<td>LsACT</td>
<td>5'-AGGTGTCATGGTGGCATGGGA-3'</td>
<td>5'-GTCTTTCAGGGCGACACG-3'</td>
</tr>
</tbody>
</table>


Ten ml methanol containing 1% (v/v) HCl was added to the sample of 1.0 g fresh weight (FW). Flavonoids in the mixture were extracted under the dark overnight at 25°C. Twenty ml chloroform: 2M HCl (1:1, v:v) solution was added to the 10 ml extract. The mixture was shaken well, and centrifuged at 2000 g for 5 min, and isolated 2 layers at 4°C. The supernatant was collected and hydrolyzed at 90°C for 90 min. The hydrolyzed sample was cooled in ice water. Five ml diethyl ether was added to about 10 ml hydrolyzed sample. The mixture was shaken well, centrifuged at 2000 g for 5 min, and isolated 2 layers. The diethyl ether layer including quercetin was collected 3 times. The pooled diethyl ether of about 15 ml was condensed using centrifugal concentrator. The desiccated residue was dissolved in 0.5 ml methanol (99.5%) for the next analysis.

**Quantification of quercetin aglycone**

Analysis of the flavon aglycone was performed using an HPLC system (LaChrom Elite, Hitachi, Japan) equipped with photo diode array detector (L2450, Hitachi, Japan) through a 4.6 mm × 150 mm CAPCELL PAK AG120 (Shiseido, Japan) at 40°C with a mobile phase comprising methanol: acetic acid (2:3, pH 2.0) solution at a flow rate of 1.5 ml min⁻¹. UV-visible spectra were recorded in the 200–700 nm range, and the flavon aglycone was detected at 370 nm. The identification of flavon aglycone was performed 3 times by a comparison of its retention time (RT) and UV-visible spectra with those of authentic standards (kaempferol, luteolin, myricetin, and quercetin, Sigma-Aldrich, USA). The absorbance spectra of the chromatographic peaks formed by the extracts of the treated plants were recorded using the HPLC system equipped with an UV/Vis detector (SPD-6AV, Shimadzu, Japan).

**RESULTS**

**Isolation of Lactuca sativa flavonol synthase gene (LsFLS)**

To analyze the level of FLS expression in red leaf lettuce, the partial sequences of LsFLS (Accession number AB359897) and LsACT (Accession number AB359898) were determined using gene-specific primers (Table 1) designed from known sequences in the database (Table 2). The deduced amino acid sequence of the lettuce FLS protein showed 74% amino acid identity with FLS from *Vitis vinifera* (60-294aa) and *Glycine max* (59-293aa), 73% with those from *Nicotiana tabacum* (70-305aa), 70% *Pyrus communis* (58-296aa), 66% *A. thaliana* (60-294aa), and 58% *Oryza sativa* (56-290aa).

**Effects of light quality on LsFLS expression in red leaf lettuce**

The qPCR was performed to quantify LsFLS expression in the young and mature leaves of red leaf lettuce. In this experiment, gene expression was observed in plants exposed to UV-B, UV-A, blue, and red light at night. Under blue light condition, the LsFLS expression in the young and mature leaves increased respectively by 4.7 times (Fig. 3A) and 1.8 times (Fig. 3B) compared with those in the respective controls. On exposure to both UV-A and blue light, the LsFLS expression in
Table 2 Comparison of the amino acid sequences of enzymes from the red leaf lettuce gene with published sequences from other species.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Botanical name</th>
<th>Area (aa)</th>
<th>Identity (%)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLS</td>
<td>Vitis vinifera</td>
<td>60–294</td>
<td>74</td>
<td>Q2PGC6</td>
</tr>
<tr>
<td>Glycine</td>
<td>max</td>
<td>59–293</td>
<td>73</td>
<td>Q6EDG6</td>
</tr>
<tr>
<td>Nicotiana</td>
<td>tabacum</td>
<td>70–305</td>
<td>73</td>
<td>Q1PHN5</td>
</tr>
<tr>
<td>Pyrus</td>
<td>communis</td>
<td>58–296</td>
<td>70</td>
<td>A0EKE7</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>thaliana</td>
<td>60–294</td>
<td>66</td>
<td>Q96330</td>
</tr>
<tr>
<td>Oryza</td>
<td>sativa</td>
<td>56–290</td>
<td>58</td>
<td>Q6Z306</td>
</tr>
<tr>
<td>ACT</td>
<td>Vitis vinifera</td>
<td>42–108</td>
<td>100</td>
<td>ASBOL2</td>
</tr>
<tr>
<td>Glycine</td>
<td>max</td>
<td>22–88</td>
<td>100</td>
<td>Q96443</td>
</tr>
<tr>
<td>Nicotiana</td>
<td>tabacum</td>
<td>42–108</td>
<td>100</td>
<td>Q6F4H4</td>
</tr>
<tr>
<td>Pyrus</td>
<td>communis</td>
<td>28–94</td>
<td>100</td>
<td>Q5CCU0</td>
</tr>
<tr>
<td>Arabidopsis</td>
<td>thaliana</td>
<td>42–108</td>
<td>100</td>
<td>P541W9</td>
</tr>
<tr>
<td>Oryza</td>
<td>sativa</td>
<td>42–108</td>
<td>100</td>
<td>Q94DL4</td>
</tr>
</tbody>
</table>

The DDBJ/EMBL/GenBank accession numbers of LsFLS and LsACT are AB359897 and AB359898, respectively.

Fig. 3 Effects of red and blue light on level of LsFLS expression. Red leaf lettuce was grown under the dark (Control), red light (60 μmol m−2 s−1) and blue light (60 μmol m−2 s−1) during the night for 2 weeks. Young (A, the third true leaves) and mature (B, the fifth true leaves) leaves of 28-day-old plants were harvested. Data are the means and SE of 3 replicate experiments. Letters indicate significance at the p < 0.05 levels determined by Turkey’s multiple range test.

the young and mature leaves increased respectively by 6.8 times (Fig. 4A) and 5.2 times (Fig. 4B) compared with that in the respective controls. On exposure to both UV-B and blue light, the LsFLS expression in the young and mature leaves increased respectively by 11.7 times (Fig. 5A) and 6.5 times (Fig. 5B) compared with that in the respective controls. When UV-B together with blue light was used, the LsFLS expression in the young leaves (Fig. 5A) increased by 21.1 times compared with that in the mature leaves (Fig. 5B).

Effects of light quality on quercetin content in the three lettuce cultivars

The relation between light quality and quercetin content in the three lettuce cultivars (red, green, and butterhead) was confirmed by HPLC analysis. When red leaf lettuce cultivated under both UV-B and blue light, the quercetin content in the young leaves (Fig. 6A, Blue+UV-B) increased by 13.4 times compared with that in the control (Fig. 6A) and by 5.7 times compared with that in the mature leaves (Fig. 6B, Blue+UV-B). In green leaf lettuce exposed to both UV-B and blue light, the quercetin content in the young leaves (Fig. 7A, Blue+UV-B) increased by 6.0 times compared with that in the control (Fig. 7A) and by 1.3 times compared with that in the mature leaves (Fig. 7B, Blue+UV-B). In butterhead lettuce subjected to the same light conditions, the quercetin content in the young leaves (Fig. 8A, Blue+UV-B) increased by 11.0 times compared
QUERCETIN CONTENT AND FLAVONOL SYNTHASE GENE EXPRESSION

![Graphs](image)

**Fig. 4** Effects of UV-A together with blue light on level of *LsFLS* expression. Red leaf lettuce was grown under the dark (Control), UV-A irradiation (9.5Wm⁻²) and blue light (60 μmol m⁻²s⁻¹) during the night for 2 weeks. Young (A, the third true leaves) and mature (B, the fifth true leaves) leaves of 28-day-old plants were harvested. Data are the means and SE of 3 replicate experiments. Letters indicate significance at the p<0.05 levels determined by Turkey’s multiple range test.

![Graphs](image)

**Fig. 5** Effects of UV-B together with blue light on level of *LsFLS* expression. Red leaf lettuce was grown under the dark (Control), UV-B irradiation (0.5Wm⁻²) and blue light (60 μmol m⁻²s⁻¹) during the night for 2 weeks. Young (A, the third true leaves) and mature (B, the fifth true leaves) leaves of 28-day-old plants were harvested. Data are the means and SE of 3 replicate experiments. Letters indicate significance at the p<0.05 levels determined by Turkey’s multiple range test.

with that in the control (Fig. 8A) and by 4.6 times compared with that in the mature leaves (Fig. 8B, Blue+UV-B).

**DISCUSSION**

Quercetin has important functions that can be used as a dietary supplement (Finley, 2005; Theoharides and Bielory, 2004). FLS is a key enzyme involved in quercetin biosynthesis (Fig. 1; Stracke et al., 2007; Pelletier et al., 1999). Since *FLS* is affected by light (Pelletier et al., 1999; Wisman et al., 1998), the relation between light quality, *LsFLS* expression and quercetin content was investigated. First, we investigated the effects of light quality on *LsFLS* expression in young and mature leaves of red leaf lettuce. Next, referring to the results of *LsFLS* expression, the leaf lettuce of three cultivars were exposed to different light using fluorescent lamps at night in other to clarify light quality that increase quercetin content.

*LsFLS* expression did not increase in experimental treatment of UV-A with blue light compared with that of only UV-A (Fig. 4). It is thought that cryptochromes absorb UV-A and blue light in *A. thaliana* (Wade et al., 2001; Jenkins, 1997; Fuglevand et al., 1996). Therefore, when irradiated UV-A with blue light, signals that increase *LsFLS* expression may not amplify.
Fig. 6 Effects of UV-B together with blue light on quercetin content in red leaf lettuce. Experimental light conditions were described in Fig. 5. Young (A, the third true leaves) and mature (B, the fifth true leaves) leaves of 28-day-old plants were harvested. Data are the means and SE of 4 replicate experiments. Letters indicate significance at the p < 0.05 levels determined by Turkey’s multiple range test.

Fig. 7 Effects of UV-B together with blue light on quercetin content in green leaf lettuce. Experimental light conditions were described in Fig. 5. Young (A, the third true leaves) and mature (B, the fifth true leaves) leaves of 28-day-old plants were harvested. Data are the means and SE of 4 replicate experiments. Letters indicate significance at the p < 0.05 levels determined by Turkey’s multiple range test.

Fig. 8 Effects of UV-B together with blue light on quercetin content in butterhead lettuce. Experimental light conditions were described in Fig. 5. Young (A, the fourth true leaves) and mature (B, the eighth true leaves) leaves of 28-day-old plants were harvested. Data are the means and SE of 4 replicate experiments. Letters indicate significance at the p < 0.05 levels determined by Turkey’s multiple range test.
QUERCETIN CONTENT AND FLAVONOL SYNTHASE GENE Expression

We showed that *LsFLS* expression increase remarkably in light condition of UV-B together with blue light compared with that of only UV-B (Fig. 5). It was predicted that UV-B level was low and/or there were not synergistically effects of blue light when plants were irradiated only UV-B at night. It is reported that UV-B and UV-A/blue light photoreceptors interact synergistically to stimulated CHS expression in *A. thaliana* (Wade et al., 2001; Jenkins, 1997; Fuglevand et al., 1996). Our results supported that *FLS* expression responds to light as well as *CHS* expression (Pellet et al., 1999; Wisman et al., 1998).

In this study, the quercetin content increased significantly when leaf lettuce was exposed to both UV-B and blue light (Figs. 6, 7, 8). It was reported that the quercetin content was increased by simultaneous exposure to UV-B and white light at daytime in *Trifolium repens* (Hofmann et al., 2003, 2001, and 2000) and in *Pinus sylvestris* (Lavola et al., 2003). It is thought that the quercetin content increase because UV-B interacts synergistically with the blue light included in white light.

The *LsFLS* expression level (Figs. 3, 4, 5) and quercetin content (Figs. 6, 7, 8) in mature leaves are extremely low compared with those in young leaves. It appears that the quercetin content in the young and mature leaves is regulated at each developmental stage. Quercetin has protective properties against UV-B (Kaffarnik et al., 2005; Turunen et al., 1999; Middleton and Teramura, 1993) in addition to antioxidant and free radical scavenging activities (Moreira et al., 2005; Lugasi et al., 2003; Choi et al., 2003; Middleton et al., 2000). It is thought that the higher quercetin accumulation in young leaves before maturing protects the lettuce leaves in early developmental stages. In *Nierembergia caerulea* petals (Ueyama et al., 2006) and *Eustoma grandiflorum* flower buds (Noda et al., 2004), *FLS* expression and the flavonol content increased during the early developmental stages (closed buds), whereas they decreased in the later developmental stages (opened flowers). Therefore, it is inferred that there are regulatory factors of *LsFLS* expression involved in the developmental stages. In *A. thaliana*, *MYB11*, *MYB12*, *MYB111*, and *FLS1* were identified as *FLS* regulatory genes because the flavonol content was reduced in *myb11*, *myb12*, and *myb111* (Stracke et al., 2007), and *fls1* mutants (Routaboul et al., 2006). Further work is required to determine whether *FLS* regulatory genes may be developmentally regulated in *Lactuca sativa* L.

We clarified effects of light quality on *FLS* expression in red leaf lettuce and quercetin content in three leaf lettuce cultivars. Our results demonstrated that *FLS* expression was positively correlated with quercetin accumulation on exposure to UV-B with blue light. We want to increase in the quercetin content of mature leaves as well as young leaves.

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