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Running title: Effect of red and blue lights on ascorbate metabolism in broccoli

Effect of red and blue LED light irradiation on ascorbate content and expression of genes related to ascorbate metabolism in postharvest broccoli

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Abstract

In the present study, the effects of red and blue light-emitting diode (LED) lights on the senescence of broccoli (*Brassica oleracea* L. var. *italica*) after harvest were investigated. The results showed that irradiation with red LED light was effective for delaying senescence in broccoli after harvest. Under red LED light, the yellowing process was delayed, and the ethylene production and reduction of ascorbate (AsA) were suppressed in broccoli after harvest. In contrast, the blue LED light treatment did not significantly affect the senescence process of broccoli after harvest. As the red light is inconvenient for the customers to select broccoli in the supermarket, we designed a type of modified white LED light. In this modified white LED light, the radio of blue light was decreased, while the radio of red light was increased. Under the modified white LED light, the AsA reduction in broccoli was slightly delayed on the first and second days after harvest. Moreover, the modulation of AsA reduction by the modified white LED light treatment was highly regulated at the transcriptional level. The up-regulation of the AsA biosynthetic genes (*BO-VTC2* and *BO-GLDH*) and AsA regeneration genes (*BO-MDAR1* and *BO-MDAR2*) contributed to the higher AsA content in the modified white LED light treatment on the first and second days after harvest. The results presented herein might provide new strategies to improve the nutritional quality of broccoli after harvest.

Key words: Ascorbate, Blue LED light, Broccoli, Modified white LED light, Red LED light, Senescence

1. Introduction

Broccoli (*Brassica oleracea* L. *var. italica*) is one of the most popular vegetables throughout the world. A number of epidemiological studies indicate that the consumption of broccoli helps to decrease the risk of development of cancers. The healthy beneficial effects of broccoli might be partly attributed to its higher contents of antioxidants, such as ascorbate (AsA), polyphenols and glucosinolates (Podsedek, 2007; Cartea and Velasco, 2008). However, fresh broccoli is a perishable immature vegetable which deteriorates or senesces rapidly after harvest at ambient temperature. During the senescence process, the florets turn yellowing and the contents of nutrition decrease rapidly to a low level (Nishikawa et al., 2003). Therefore, it is of crucial importance to prevent broccoli florets senescence during the postharvest storage. In the recent years, various techniques for delaying postharvest senescence of broccoli have been investigated, such as a modified atmosphere (MA) or controlled atmosphere (CA) (Fernández-León et al., 2013), different types of packaging (Hansen et al., 2001; Toivonen and DeEll, 2001), treatment with chemicals and cytokinins (Wang, 1977; Rushing, 1990; Downs et al., 1997), and ethanol vapour treatment (Suzuki et al., 2004). In the previous study, we found that the applications of 1-methylcyclopropene (1-MCP) and electrostatic atomization were effective to delay yellowing and decay, alleviate certain ethylene-induced postharvest physiological disorders, and suppress the reduction of AsA in broccoli after harvest (Ma et al., 2009, 2010, 2012a).

AsA, also known as the reduced form of vitamin C, plays an important role in the antioxidative defense system of plants, contributes to the regulation of cell division and

expansion, and controls the commencement of senescence (Mittler, 2002; Mori et al., 2009). As humans can not synthesize or store the AsA in the body, the fruits and vegetables are the primary sources of AsA intake for humans. Broccoli is rich in AsA, and it is a good source of AsA in daily diet. In broccoli, the AsA metabolism is complex, which is controlled by the synthesis, oxidation, and recycling processes (Fig. 1). In the past decade, the genes related to AsA metabolism have been isolated and well characterized in broccoli after harvest (Nishikawa et al., 2003; Ma et al., 2012a). Moreover, the regulation of gene expression appeared to be an important mechanism by which AsA metabolism is regulated during the senescence process in broccoli (Nishikawa et al., 2003; Ma et al., 2010, 2012a).

Light is one of the most essential environmental factors for plants. It has been reported that light prolonged 3d shelf-life and preserved nutritional quality of fresh-cut broccoli (Zhan et al., 2012). Recently, UV-B and UV-C irradiations have been shown to be useful non-chemical methods to delay floret yellowing and chlorophyll degradation in broccoli after harvest (Costa et al., 2006; Aiamla-or et al., 2009, 2010). Moreover, the combination of UV-C and hot air was effective to increase the levels of phenolics and ascorbic acid, and enhance the activity of enzymes involved in removing reactive oxygen species (ROS; Lemoine et al., 2010). In higher plants, sensing of red and blue lights is carried out by different light photoreceptors (Briggs et al., 2001). Thus, red and blue lights exhibit different effects on plant development and biosynthesis of cell components. Red light is important to the development of the photosynthetic apparatus and increases flowering, budding and starch accumulation in

plants (Saebo et al., 1995; Wu et al., 2007). Blue light regulates many plant responses including stomata opening, leaf expansion and biomass production (Xu et al., 2012; Lin et al., 2013). In citrus fruits, red light was effective to enhance carotenoid content, especially the content of β-cryptoxanthin, while blue light had no significant effect on the carotenoid content in the flavedo of Satsuma mandarin (Ma et al., 2012b). To date, however, information on the effects of red and blue lights on AsA metabolism in broccoli after harvest is still limited. In the present study, the effects of red (660 nm) and blue (470 nm) light-emitting diode (LED) lights as well as a modified white LED light on the AsA content were investigated in broccoli after harvest. Moreover, the expression of genes (*BO-VTC1*, *BO-VTC2*, *BO-GLDH*, *BO-APX1*, *BO-APX2*, *BO-sAPX*, *BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR*), which were related to the AsA metabolism, were analyzed in the broccoli treated with the modified white LED light. This study is the first of their kind to investigate effects of the light quality on the AsA metabolism of broccoli. These results will provide a new effective method to enhance the nutritional quality of broccoli after harvest.

2. Materials and Methods

2.1. Plant materials and treatments

Broccoli (*Brassica oleracea* L. var. *italica*) plants were grown at the Green Field Farm, Hamamatsu, Japan. Experimental I: Mature broccoli heads of uniform size, shape, and maturity were selected and irradiated with blue and red LED lights for four days at 20 °C. Experimental II: A type of modified white LED light was designed.

Mature broccoli heads of uniform size, shape, and maturity were selected and irradiated with the modified white light for four days at 20 °C. For the two experiments, the heads irradiated with white LED light were used as the control. LED lights utilized in this research were provided by Stanley Electric Co., Ltd, Japan. The blue and red LED lights had an arrow output spectrum with a peak of 470 nm and 660 nm, respectively. The wavelength of the white LED light in the control was from 430 nm to 730 nm. The wavelength of the modified white LED light was also from 430 nm to 730 nm. However, in the modified white LED light the radio of blue light was decreased, while the radio of red light was increased. The flow of current of each LED light was maintained by using external transformer having the output voltage of 40 V and input voltage of 100-240 V providing current of 0.35 A. The photosynthetic photon flux at the top of plants was 50 μmol $m^{-2}s^{-1}$ in all light treatments. All treatments were conducted at 20 °C under highly humidified conditions (RH > 95%). Florets were excised from the heads with a single-edged razor every day after harvest, and three replicates of three heads of broccoli were taken at each sampling time. The excised florets were immediately frozen in liquid nitrogen except for the samples for ethylene production analysis, and stored at -80 °C until used.

2.2. Assessment of broccoli yellowing

The colour of florets in broccoli was scored by a visual assessment of changes from green to yellow. A rating scale of senescence from 5 to 0 was adopted: 5, all green; 4, 20% yellowing; 3, 40% yellowing; 2, 60% yellowing; and 1, 80% yellowing. Intermediate numbers were assigned where appropriate according to the yellowing

rate.

2.3. Measurements of ethylene production

A 1-g sample of florets was placed into a 15-mL vial, the vial was sealed using a silicon rubber cap, and sample was incubated for 30 min at 20 °C. The headspace gas in the vial was sampled using a 1-mL plastic hypodermic syringe and injected into a gas chromatograph (Hitachi 163) for ethylene. The rate of ethylene production was expressed as nL ethylene per h per g FW.

2.4. Extraction and assays of AsA and DHA

The contents of AsA and DHA were assayed by HPLC. Each frozen sample was homogenized using a mortar and pestle in 10 volumes of extractant solution (3% metaphosphoric acid and 8% acetic acid). The homogenate was centrifuged at 14,000×g for 20 min, and then the supernatant was filtered through Miracloth (Calbiochem). The pH of the filtrate was adjusted by adding an equal volume of 0.2 M potassium-phosphate buffer (pH 7.5). Total amount of AsA and DHA was assayed by adding 0.5 mL of 6 mM dithiothreitol (DTT) to 0.1 mL of aliquot of filtrate and incubated in the dark at 30 °C for 15 min. After the sample was filtered through a 0.22-μm cellulose acetate filter (Advantec), a 20 μL aliquot was injected onto a J'sphere ODS-M80 column (YMC) attached to a LC-10AD pump (Shimadzu). The column kept at 20 °C was eluted with 1.5% ammonium dihydrogen phosphate (pH 3.8) at a flow rate of 1.0 mL min^{-1}. The absorbance at 245 nm (retention time 2.6 min) was monitored using an SPD-10A spectrophotometric detector (Shimadzu) attached to a chart recorder (C-R6A, Shimadzu). Peaks were converted to concentrations by using

the dilution of stock ascorbic to construct a standard curve. AsA content was determined in a similar manner without the addition of DTT. DHA content was calculated by subtraction the AsA value from the total amount.

2.5. Total RNA extraction and real-time quantitative RT-PCR

Total RNA was extracted from the florets of broccoli after harvest according to the method described by Kato et al. (2000). The total RNA was cleaned up using the RNeasy Mini Kit (Qiagen, Hilden, Germany) with on-column DNase digestion. The reverse transcription reactions were performed with 2 μg of purified RNA and a random hexamer at 37 °C for 60 min using TaqMan Reverse Transcription Reagents (Applied Biosystems).

TaqMan MGB probes and sets of primers for *BO-VTC1*, *BO-VTC2*, *BO-GLDH*, *BO-APX1*, *BO-APX2*, *BO-sAPX*, *BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR* were designed with the Primer Express software (Applied Biosystems; Ma et al., 2012a). For endogenous control, the TaqMan Ribosomal RNA Control Reagents VIC Probe (Applied Biosystems) was used. TaqMan real-time PCR was carried out with the TaqMan Universal PCR Master Mix (Applied Biosystems) using StepOnePlus™ Real-Time PCR System (Applied Biosystems) according to the manufacture's instructions. Each reaction contained 900 nM primers, a 250 nM TaqMan MGB Probe, and template cDNA. The thermal cycling conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The levels of gene expression were analyzed with StepOnePlus™ Real-Time PCR System Software (Applied Biosystems) and normalized with the results of 18S ribosomal RNA. Real-time quantitative

RT-PCR was performed in three replicates for each sample.

2.6. Statistical analysis

All values are shown as the mean ± SE for three replicates. The data were analyzed using Tukey's HSD test and Student's *t*-test ($P < 0.05$).

3. Results

3.1 Effects of red and blue LED lights on yellowing, ethylene production and AsA content

In the present study, we observed that florets showed significant yellowing after two days, and on the fourth day, the florets was complete yellowing in the control at 20 °C. Thus, we preserved the broccoli for four days in the present research. Compared with the control, the yellowing process was delayed by the red LED treatment, but it was not affected by the blue LED light treatment. On the third day, the florets displayed obvious yellowing in the control and blue LED light treatment, while in the red LED light treatment most of the florets remained green. Ethylene production increased with a peak on the third day after harvest in the control. Under red LED light, ethylene production decreased slightly on the first day, and then increased rapidly. The ethylene production in the red LED light treatment was much lower than that of the control (Fig. 2B). Under blue LED light, ethylene production increased significantly from the first day. Compared with the control, the ethylene production in the blue LED light treatment was not significantly affected (Fig. 2B).

AsA content decreased rapidly after harvest in the control (Fig. 3). Under red LED

light, the reduction of AsA content was clearly slowed. On the first and second days, AsA content in the red LED light treatment was higher than that of the control. Under blue LED light, AsA content was similar to that in the control throughout the experiment period. In broccoli, the content of DHA was relatively low, showing less than 10% of total ascorbate. The content of DHA kept almost constant in the control, and it was not significantly affected by the red and blue LED lights.

Taken together, these results suggested that red LED light was effective in delaying the broccoli senescence after harvest, while the blue LED light can not significantly affect the senescence process in broccoli.

3.2 Effects of the modified white LED light on AsA content

In the supermarket, it is not convenient for the customers to select the broccoli under the red light. Thus, we designed a type of modified white LED light, in which the radio of blue light was decreased, while the radio of red light was increased (Fig. 4). The yellowing process and ethylene production were not significantly affected by the modified white LED light (Supplementary Fig. 1). In contrast, the reduction of AsA content after harvest was slightly delayed by the modified white LED light (Fig. 5). Compared with the control, AsA content was higher in the modified white LED light treatment on the first and second days after harvest.

3.3 Effects of the modified white LED light on expression of genes related to AsA metabolism

In the present study, the changes in the expression of AsA biosynthetic genes (*BO-VTC1*, *BO-VTC2*, and *BO-GLDH*), AsA breakdown genes (*BO-APX1*, *BO-APX2*,

and *BO-sAPX*), and AsA regeneration genes (*BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR*) were investigated (Fig. 1). In the control, the expression of *BO-VTC1* and *BO-GLDH* decreased after harvest, and then increased slightly. The expression of *BO-VTC2* increased gradually after harvest in the control. Under the modified white LED light, the expression of *BO-VTC2* and *BO-GLDH* was up-regulated on the first and second days. The expression of *BO-VTC1* was up-regulated on the second day after harvest by the modified white LED light (Fig. 6).

The expression of the two cytosolic APX genes, *BO-APX1* and *BO-APX2*, increased gradually after harvest in the control. The expression of *BO-sAPX*, which is the stromal APX in chloroplast, decreased rapidly after harvest, and then increased slightly in the control. Under the modified white LED light, the expression of *BO-APX1* was up-regulated on the first and second days. The expression of *BO-APX2* was down-regulated on the first day, and it was up-regulated on the second day by the modified white LED light. The expression of *BO-sAPX* was up-regulated on the first day after harvest by the modified white LED light (Fig. 6).

The expression of *BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR* decreased after harvest and then increased slightly in the control. Under the modified white LED light, the expression of *BO-MDAR1* and *BO-MDAR2* was up-regulated on the first and second days after harvest. The expression of *BO-DHAR* was down-regulated on the first day, and it was not affected on the second day by the modified white LED light treatment (Fig. 6).

4. Discussion

In higher plants, sensing of red and blue lights is carried out by different photoreceptors. Red light is absorbed by phytochromes family, whereas blue light is absorbed by cryptochrome and phototropin photoreceptors (Lin, 2000; Bohne and Linden, 2002). Thus, red and blue lights produce different morphogenetic and photosynthetic responses in plants. In tomato, illumination with blue LED light was effective in fruit yield increase and quality improvement as well as improvement in disease resistance, while illumination with red LED light induced the accumulation of lycopene along with an increase in total carotenoid content (Alba et al., 2000; Schofield and Paliyath, 2005; Liu et al., 2009; Xu et al., 2012). In the previous study, we reported that red LED light was effective to enhance carotenoid content, especially the content of β-cryptoxanthin, while blue LED light had no significant effect on the carotenoid content in the flavedo of citrus fruits (Ma et al., 2012b). Fresh broccoli is a perishable immature vegetable which deteriorates or senesces rapidly after harvest at ambient temperature. During storage, the appearances of yellowing and ethylene climacteric peak are direct indexes for senescence in broccoli. Therefore, it is crucial to delay the yellowing and suppress the ethylene production of broccoli during post-harvest storage (Suzuki et al., 2004; Ma et al., 2009, 2012a). It has been reported that UV-B and UV-C treatments effectively delayed floret yellowing and chlorophyll degradation by suppressing the activities of chlorophyll-degrading enzymes in broccoli after harvest, indicating that UV-B and UV-C irradiations were useful methods to suppress the senescence and maintain the quality of broccoli after harvest (Costa et al.,

2006; Aiamla-or et al., 2009, 2010). However, in the UV-A treatment the yellowing process was not inhibited in broccoli after harvest (Aiamla-or et al., 2009). In the present study, the results showed that red LED light delayed the yellowing process and decreased the ethylene production of broccoli after harvest. In contrast, the yellowing process and ethylene production were not significantly affected by the blue LED light treatment. These results suggested that irradiation with red LED light was effective for delaying senescence in broccoli after harvest.

Fresh broccoli, which is rich in AsA, is a good source of AsA intake for humans. However, the content of AsA decreased rapidly to a low level in broccoli after harvest. In the recent years, various researches into the improvement of AsA level in broccoli after harvest has been performed (Shigenaga et al., 2005; Mori et al., 2009; Ma et al., 2010, 2012a). Lemoine et al., (2010) reported that the combination of UV-C and hot air can delay the loss of AsA during the postharvest of broccoli. In the previous study, we found that the applications of 1-MCP and electrostatic atomization were effective methods to suppress the AsA loss in broccoli (Ma et al., 2010, 2012a). In the present study, the results showed that the blue LED light treatment did not affect the AsA metabolism, while the red LED light treatment was effective to improve the nutritional value of broccoli by delaying the AsA reduction after harvest. In addition, AsA as a critical antioxidative component is effective to scavenge ROS, which is closely related with the senescence of fruits and vegetables. In the present study, the higher AsA level in the red LED light treatment indicated that red LED light enhanced the antioxidant capability to scavenge ROS, which might lead to delay the senescence of broccoli after

harvest.

Although red LED light is effective to delay the senescence and maintain the nutritional value of broccoli after harvest, it is inconvenient for the customers to select the broccoli in the supermarket under the red light. Thus, in the present study we designed a type of modified white LED light. In this kind of modified white LED light, the radio of blue light was decreased, while the radio of red light was increased (Fig. 4). Compared with the control, the reduction of AsA in broccoli after harvest was delayed by the modified white LED light. The AsA content was higher in the modified white LED light treatment than that of the control on the first and second days after harvest. However, under the modified white LED light, the yellowing process and ethylene production were not significantly suppressed, indicating that the senescence process was not delayed by the modified white LED light in broccoli after harvest. Thus, to enhance the efficacy of the modified white LED light, more research is still needed in the future, including (i) further increase the radio of the red light in the modified white LED light; (ii) increase the intensity of modified white LED light; (iii) change the operational mode of the modified white LED light, such as pulsed irradiation.

In broccoli, AsA accumulation is controlled by biosynthesis, oxidation and regeneration processes. In the past decade, the genes related to AsA metabolism have been cloned, and their expression profiles have been extensively investigated in broccoli after harvest (Nishikawa et al., 2003; Ma et al., 2012a). Nishikawa et al., (2003) reported that the regulation of gene expression appeared to be an important mechanism by which AsA metabolism was regulated during the senescence process in

broccoli. In the previous study, we found that the suppression of the AsA reduction by 1-MCP and electrostatic atomization was highly regulated at the transcriptional level in broccoli after harvest (Ma et al., 2010, 2012a). In the present study, the results showed that the expression of *BO-VTC2* and *BO-GLDH* was up-regulated on the first and second days, and the expression of *BO-VTC1* was up-regulated on the second day after harvest by the modified white LED light. The up-regulation of *BO-VTC2* and *BO-GLDH*, which are important AsA biosynthetic genes, was consistent with the higher AsA content in broccoli treated by the modified white LED light on the first and second days after harvest. In higher plants, APX is the key enzyme responsible for AsA oxidation. In broccoli, two cytosolic APX genes, *BO-APX1* and *BO-APX2* were identified. Under the modified white LED light, the expression of *BO-APX1* was up-regulated on the first and second day after harvest. The expression of *BO-APX2* was down-regulated on the first day, while it was up-regulated on the second day by the modified white LED light. The expression of *BO-sAPX*, which is a chloroplast APX, was up-regulated on the first day, and was similar to the control on the second day. The regulation of expression of *BO-APX1*, *BO-APX2*, and *BO-sAPX* was not well correlated with the higher AsA content on the first and second days, suggesting that regulation of APX at the transcriptional level might not play a crucial role in suppression of AsA reduction in response to the modified white LED light treatment in broccoli after harvest. The similar results were also observed in the broccoli treated by the electrostatic atomization (Ma et al., 2012a). MDAR and DHAR are two enzymes responsible for AsA regeneration in plants (Wheeler et al., 1998; Mittler, 2002; Apel

and Hirt, 2004; Sairam and Tyagi, 2004). With the treatment of electrostatic atomization, the up-regulation of the gene expression of *BO-DHAR*, *BO-MDAR1*, and *BO-MDAR2* contributed to the suppression of AsA reduction in broccoli after harvest (Ma et al., 2012a). In the present study, the gene expression of *BO-DHAR* was down-regulated by the modified white LED light, which was not correlated with the higher AsA content. In contrast, the up-regulation of the gene expression of *BO-MDAR1* and *BO-MDAR2* was well consistent with the higher AsA content in broccoli treated by the modified white LED light on the first and second days after harvest. Taken together, these results suggested that the suppression of AsA reduction by the modified white LED light treatment was attributed to the up-regulation of the AsA biosynthetic genes (*BO-VTC2* and *BO-GLDH*) and AsA regeneration genes (*BO-MDAR1* and *BO-MDAR2*).

5. Conclusion

These results showed that irradiation with red LED light was effective for delaying senescence of broccoli after harvest, while the blue LED light treatment did not significantly affect the senescence process of broccoli after harvest. In the modified white LED light treatment, the yellowing process and ethylene production were not suppressed, while the AsA reduction was slightly delayed in broccoli on the first and second days after harvest. Moreover, the modulation of AsA reduction by the modified white LED light was highly regulated at the transcriptional level. The results presented in this study might provide new methods to improve the nutritional and commercial

values of fruits and vegetables after harvest.

Acknowledgement

This work was supported by the Feasibility study of Adaptable and Seamless Technology transfer Program through target-driven R&D (A-STEP) from Japan Science and Technology Agency (JST)

Appendix A. Supplementary data

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Figure legends

Fig. 1. The main pathway of ascorbate metabolism in plants. VTC1, GDP-D-mannose pyrophosphorylase; VTC2, GDP-L-galactose phosphorylase; GLDH, L-galactono-1,4-lactone dehydrogenase; AsA, ascorbate; APX, ascorbate peroxidase; MDA, monodehydroascorbate; MDAR, monodehydroascorbate reductase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase.

Fig. 2. Effect of red and blue LED lights on colour score (A) and ethylene production (B) of broccoli heads. The results shown are the mean ± SE for triplicate samples. Means denoted by the same letter did not differ significantly at $P < 0.05$ according to Tukey's HSD test. Some error bars and symbols are hidden by symbols.

Fig. 3. Effect of red and blue LED lights on AsA content of broccoli heads. The results shown are the mean ± SE for triplicate samples. Means denoted by the same letter did not differ significantly at $P < 0.05$ according to Tukey's HSD test. Some error bars and symbols are hidden by symbols.

Fig. 4. Spectral profiles of the red LED light, blue LED light, white LED light (control), and modified white LED light used in this study.

Fig. 5. Effect of the modified white LED light on AsA content of broccoli heads. The results shown are the mean ± SE for triplicate samples. Means denoted by the same letter did not differ significantly at $P < 0.05$ according to Student's *t*-test. Some error bars and symbols are hidden by symbols.

Fig. 6. Effect of the modified white light on the expression of genes related to the AsA metabolism in broccoli. The mRNA levels were analyzed by TaqMan real-time

quantitative RT-PCR. Real-time RT-PCR amplification of 18S ribosomal RNA was used to normalize the expression of the genes under identical conditions. The results shown are the mean ± SE for triplicate samples. Means denoted by the same letter did not differ significantly at $P < 0.05$ according to Student's *t*-test. Some error bars and symbols are hidden by symbols.

Fig. 1

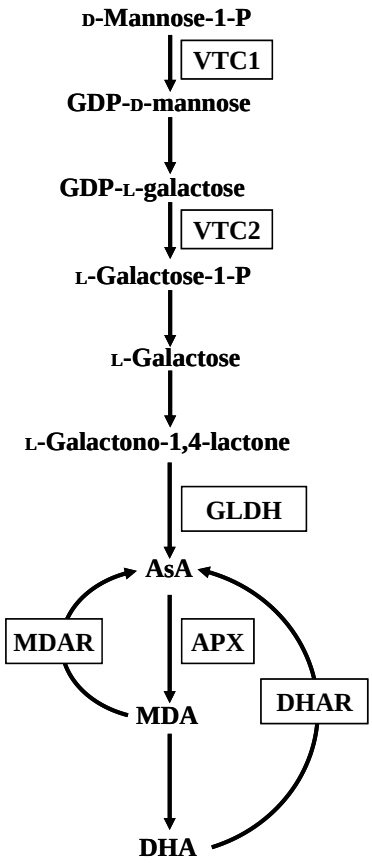

D-Mannose-1-P
VTC1
GDP-D-mannose
GDP-L-galactose
VTC2
L-Galactose-1-P
L-Galactose
L-Galactono-1,4-lactone
GLDH
AsA
MDAR
APX
DHAR
MDA
DHA

Fig. 2

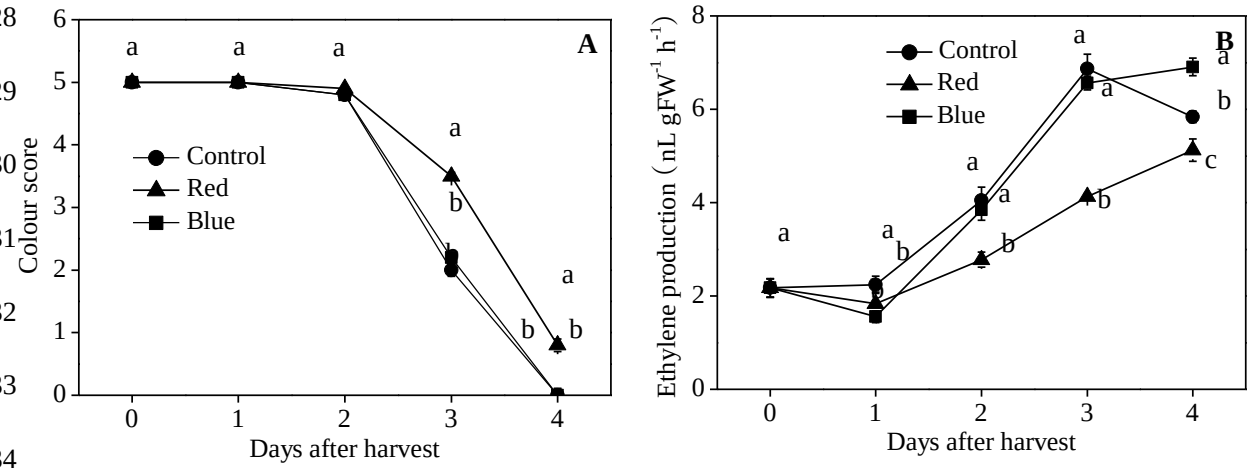

A
Colour score
Days after harvest
Control
Red
Blue
B
Ethylene production (nL gFW-1 h-1)
Days after harvest
Control
Red
Blue

Fig. 3

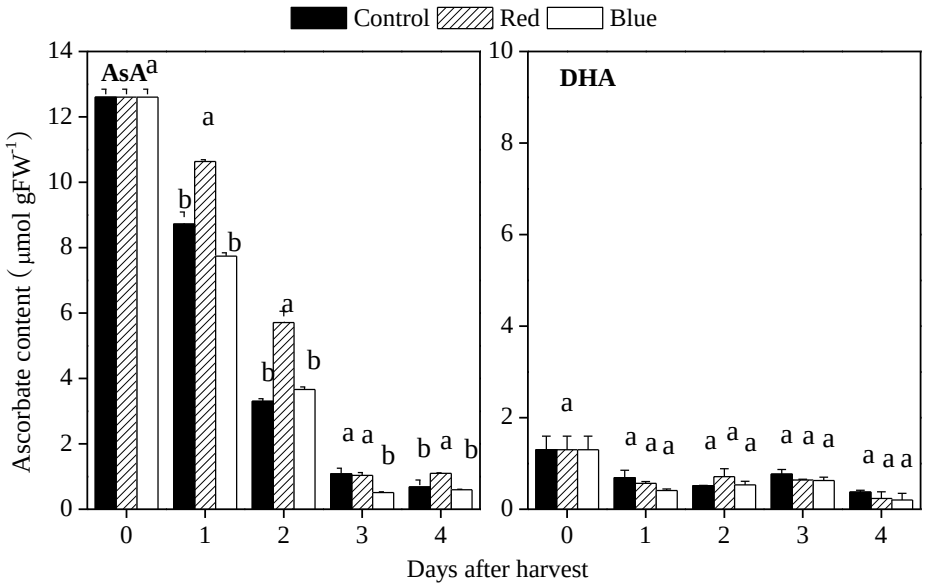

Control
Red
Blue
AsA
DHA
Ascorbate content (μmol gFW-1)
Days after harvest

Fig. 4

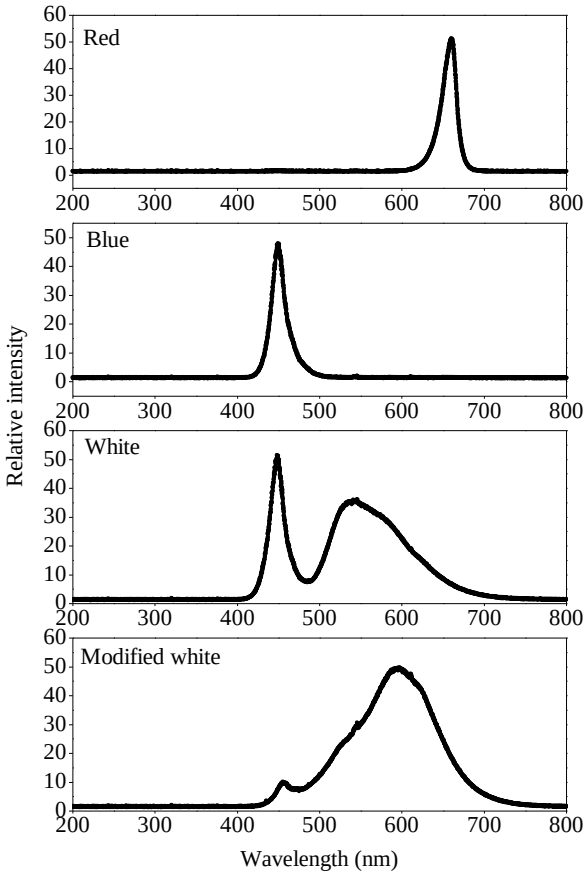

Red
Blue
White
Modified white
Relative intensity
Wavelength (nm)
0
10
20
30
40
50
60
200
300
400
500
600
700
800

Fig. 5

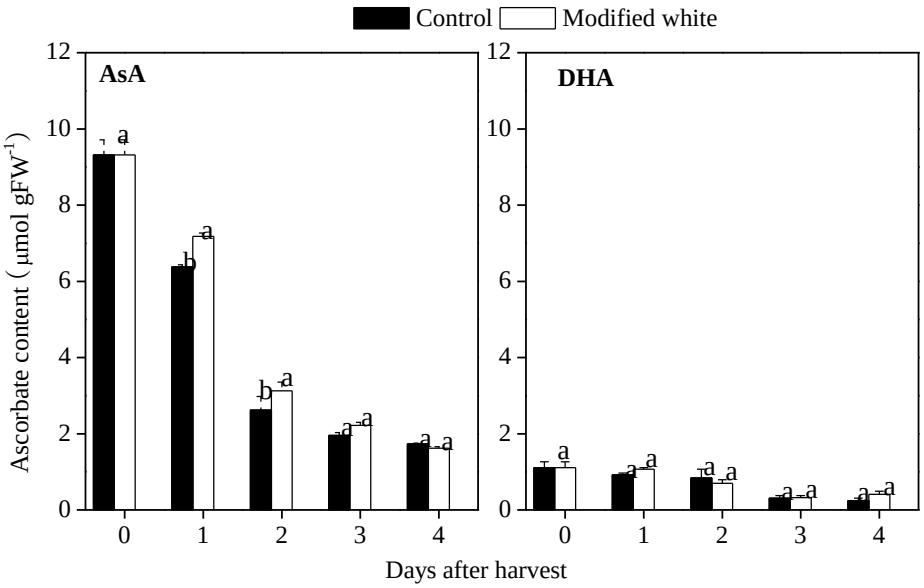
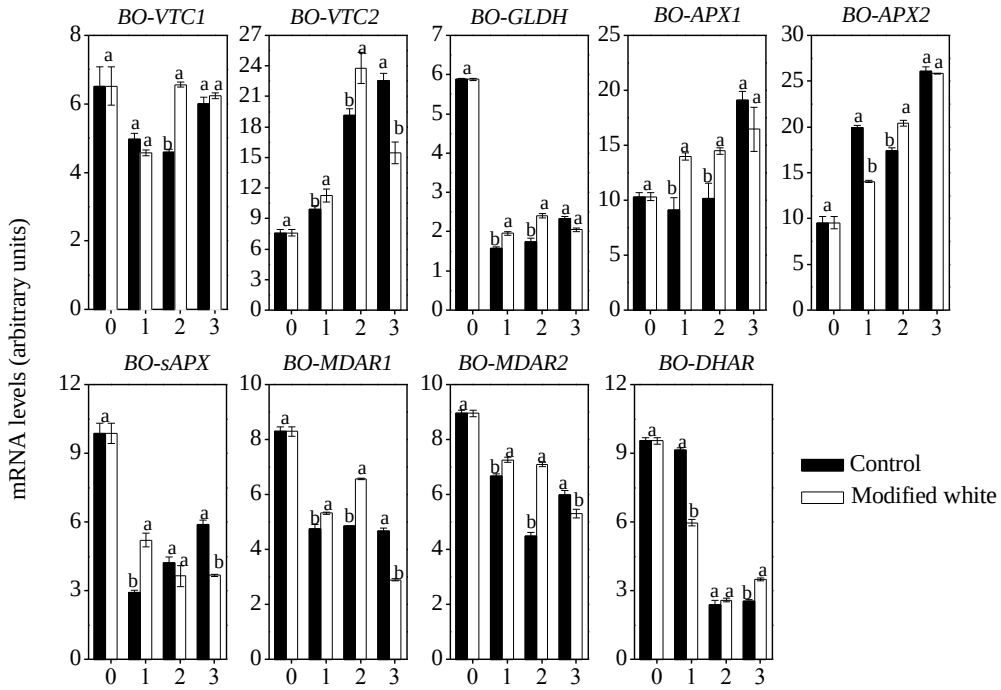

Control
Modified white
AsA
DHA
Ascorbate content (μmol gFW-1)
Days after harvest
0
1
2
3
4
6
8
10
12
a
b

Fig. 6


BO-VTC1
BO-VTC2
BO-GLDH
BO-APX1
BO-APX2
BO-sAPX
BO-MDAR1
BO-MDAR2
BO-DHAR
mRNA levels (arbitrary units)
Control
Modified white