1 **Running title: Effect of red and blue light on ascorbate metabolism in broccoli**
2
3 **Effect of red and blue LED light irradiation on ascorbate content and**
4 **expression of genes related to ascorbate metabolism in postharvest broccoli**
5 Gang Ma^a, Lancui Zhang^a, Chandra Kurnia Setiawan^{a, #}, Kazuki Yamawaki^a,
6 Tatsuo Asai^a, Fumie Nishikawa^b, Shigenori Maezawa^c, Hiroshi Sato^d, Nobuhisa
7 Kanemitsu^e, Masaya Kato^{a,*}
8 *^aDepartment of Biological and Environmental Sciences, Faculty of Agriculture,*
9 *Shizuoka University, 836 Ohya, Suruga, Shizuoka 422-8529, Japan*
10 *^bDepartment of Citrus Research, National Institute of Fruit Tree Science,*
11 *Kuchinotsu, Nagasaki 859-2501, Japan*
12 *^cThe United Graduate School of Agricultural Science, Gifu University, 1-1*
13 *Yanagido, Gifu 501-1193, Japan*
14 *^dStanley Electric Co.,Ltd. Resarch & Development Center, 1-3-1 Edanishi, Aoba,*
15 *Yokohama, Kanagawa 225-0014, Japan*
16 *^eStanley Electric Co.,Ltd. Yokohama Technical Center, 2-14-1 Edanishi, Aoba,*
17 *Yokohama, Kanagawa 225-0014, Japan*
18 ***Corresponding author: Masaya Kato**
19 Department of Biological and Environmental Sciences, Faculty of Agriculture,
20 Shizuoka University, 836 Ohya, Suruga, Shizuoka 422-8529, Japan
21 Telephone: 81-54-238-4830 Fax: 81-54-238-4830
22 E-mail address: amkato@ipc.shizuoka.ac.jp
23 [#] Present address: Lecturer in Agrotechnology Departement, Agriculture Faculty,
24 Muhammadiyah University of Yogyakarta, Yogyakarta 55183, Indonesia

25 **Abstract**

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

44 **Key words:** Ascorbate, Blue LED light, Broccoli, Modified white LED light, Red
45 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 (Podsedeck, 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 nutritional contents decrease rapidly to a low level (Nishikawa et al., 2003). Therefore, it is of crucial importance to prevent broccoli floret senescence during postharvest storage. In recent years, various techniques for delaying postharvest senescence of broccoli have been investigated, such as 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 in delaying yellowing and decay, alleviating certain ethylene-induced postharvest physiological disorders, and suppressing 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 AsA in the body, fruit and

71 vegetables are the primary sources of AsA intake for humans. Broccoli is rich in AsA,
72 and it is a good source for the daily diet. In broccoli, AsA metabolism is complex,
73 controlled by synthesis, oxidation, and recycling processes (Fig. 1). In the past decade,
74 the genes related to AsA metabolism have been isolated and well characterized in
75 broccoli after harvest (Nishikawa et al., 2003; Ma et al., 2012a). Moreover, the
76 regulation of gene expression appears to be an important mechanism by which AsA
77 metabolism is regulated during the senescence process in broccoli (Nishikawa et al.,
78 2003; Ma et al., 2010, 2012a).

79 Light is one of the most essential environmental factors for plants. It has been
80 reported that light prolongs shelf-life by 3 days and preserves nutritional quality of
81 fresh-cut broccoli (Zhan et al., 2012). Recently, UV-B and UV-C irradiations have
82 been shown to be useful non-chemical methods to delay floret yellowing and
83 chlorophyll degradation in broccoli after harvest (Costa et al., 2006; Aiamla-or et al.,
84 2009, 2010). Moreover, the combination of UV-C and hot air was effective to increase
85 the levels of phenolics and ascorbic acid, and enhance the activity of enzymes
86 involved in removing reactive oxygen species (ROS; Lemoine et al., 2010). In higher
87 plants, sensing of red and blue light is carried out by different light photoreceptors
88 (Briggs et al., 2001). Thus, red and blue light exhibit different effects on plant
89 development and biosynthesis of cell components. Red light is important for the
90 development of the photosynthetic apparatus and increases flowering, budding and
91 starch accumulation in plants (Saebo et al., 1995; Wu et al., 2007). Blue light
92 regulates many plant responses including stomatal opening, leaf expansion and
93 biomass production (Xu et al., 2012; Lin et al., 2013). In citrus fruit, red light was
94 effective in enhancing carotenoid content, especially the content of β -cryptoxanthin,
95 while blue light had no significant effect on the carotenoid content in the flavedo of

96 Satsuma mandarin (Ma et al., 2012b). To date, however, information on the effects of
97 red and blue light on AsA metabolism in broccoli after harvest is still limited. In the
98 present study, the effects of red (660 nm) and blue (470 nm) light-emitting diode
99 (LED) lights as well as a modified white LED light on the AsA content were
100 investigated in broccoli after harvest. Moreover, the expression of genes (*BO-VTC1*,
101 *BO-VTC2*, *BO-GLDH*, *BO-APX1*, *BO-APX2*, *BO-sAPX*, *BO-MDAR1*, *BO-MDAR2*,
102 and *BO-DHAR*) related to AsA metabolism, were analyzed in the broccoli treated with
103 the modified white LED light. This study is the first of its kind to investigate effects
104 of the light quality on AsA metabolism of broccoli. These results will provide a new
105 effective method to enhance the nutritional quality of broccoli after harvest.

106

107 **2. Materials and Methods**

108 *2.1. Plant materials and treatments*

109 Broccoli (*Brassica oleracea* L. var. *italica*) plants were grown at the Green Field
110 Farm, Hamamatsu, Japan. Experiment I: Mature broccoli heads of uniform size, shape,
111 and maturity were selected and irradiated with blue and red LED light for four days at
112 20 °C. Experiment II: A type of modified white LED light was designed. Mature
113 broccoli heads of uniform size, shape, and maturity were selected and irradiated with
114 the modified white light for four days at 20 °C. For the two experiments, the heads
115 irradiated with white LED light were used as the control. LED lights utilized in this
116 research were provided by Stanley Electric Co., Ltd, Japan. The blue and red LED
117 lights had a narrow output spectrum with peaks of 470 nm and 660 nm, respectively.
118 The wavelengths of both the white and the modified white LED lights in the control
119 were from 430 nm to 730 nm. However, in the modified white LED light the ratio of
120 blue light was decreased, while the ratio of red light was increased. The flow of

121 current of each LED light was maintained by using external transformer having the
122 output voltage of 40 V and input voltage of 100-240 V providing current of 0.35 A.
123 The photosynthetic photon flux at the top of the plants was $50 \mu\text{mol m}^{-2}\text{s}^{-1}$ in all light
124 treatments. All treatments were conducted at 20 °C under highly humidified
125 conditions (RH > 95%).

126 Florets were excised from the heads with a single-edged razor every day after
127 harvest, and three replicates of three heads of broccoli were taken at each sampling
128 time. The excised florets were immediately frozen in liquid nitrogen except for the
129 samples for ethylene production analysis, and stored at -80 °C until used.

130 *2.2. Assessment of broccoli yellowing*

131 The colour of florets in broccoli was scored by a visual assessment of changes from
132 green to yellow. A rating scale of senescence from 5 to 0 was adopted: 5, all green; 4,
133 20% yellowing; 3, 40% yellowing; 2, 60% yellowing; and 1, 80% yellowing.
134 Intermediate numbers were assigned where appropriate according to the yellowing
135 rate.

136 *2.3. Measurements of ethylene production*

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

142 *2.4. Extraction and assays of AsA and DHA*

143 The contents of AsA and DHA were assayed by HPLC. Each frozen sample was
144 homogenized using a mortar and pestle in 10 volumes of extractant solution (3%
145 metaphosphoric acid and 8% acetic acid). The homogenate was centrifuged at

146 14,000×g for 20 min, and then the supernatant was filtered through Miracloth
147 (Calbiochem). The pH of the filtrate was adjusted by adding an equal volume of 0.2 M
148 potassium-phosphate buffer (pH 7.5). Total amount of AsA and DHA was assayed by
149 adding 0.5 mL of 6 mM dithiothreitol (DTT) to 0.1 mL of aliquot of filtrate and
150 incubated in the dark at 30 °C for 15 min. After the sample was filtered through a
151 0.22-µm cellulose acetate filter (Advantec), a 20 µL aliquot was injected into a
152 J'sphere ODS-M80 column (YMC) attached to a LC-10AD pump (Shimadzu). The
153 column kept at 20 °C was eluted with 1.5% ammonium dihydrogen phosphate (pH 3.8)
154 at a flow rate of 1.0 mL min⁻¹. The absorbance at 245 nm (retention time 2.6 min) was
155 monitored using an SPD-10A spectrophotometric detector (Shimadzu) attached to a
156 chart recorder (C-R6A, Shimadzu). Peaks were converted to concentrations by using
157 the dilution of stock ascorbate to construct a standard curve. AsA content was
158 determined in a similar manner without the addition of DTT. DHA content was
159 calculated by subtracting the AsA value from the total amount.

160 2.5. Total RNA extraction and real-time quantitative RT-PCR

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

167 TaqMan MGB probes and sets of primers for *BO-VTC1*, *BO-VTC2*, *BO-GLDH*,
168 *BO-APX1*, *BO-APX2*, *BO-sAPX*, *BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR* were
169 designed with the Primer Express software (Applied Biosystems; Ma et al., 2012a).
170 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 manufacturer'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 $\pm$ 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

We observed that florets showed significant yellowing after two days, and on the fourth day, the florets had complete yellowing in the control at 20 °C; thus, we preserved the broccoli for four days. 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 down. 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 remained almost constant in the control, and was not significantly affected by the red and blue LED lights.

Taken together, these results suggested that red LED light was effective in delaying broccoli senescence after harvest, while the blue LED light did 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 customers to select broccoli under the red light. Thus, we designed a type of modified white LED light, in which the ratio of blue light was decreased, while the ratio 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

221 The changes in the expression of AsA biosynthetic genes (*BO-VTC1*, *BO-VTC2*,
222 and *BO-GLDH*), AsA breakdown genes (*BO-APX1*, *BO-APX2*, and *BO-sAPX*), and
223 AsA regeneration genes (*BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR*) were
224 investigated (Fig. 1). In the control, the expression of *BO-VTC1* and *BO-GLDH*
225 decreased after harvest, and then increased slightly. The expression of *BO-VTC2*
226 increased gradually after harvest in the control. Under the modified white LED light,
227 the expression of *BO-VTC2* and *BO-GLDH* was up-regulated on the first and second
228 days. The expression of *BO-VTC1* was up-regulated on the second day after harvest
229 by the modified white LED light (Fig. 6).

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

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

244

245

246 **4. Discussion**

247 In higher plants, sensing of red and blue lights is carried out by different
248 photoreceptors. Red light is absorbed by members of the phytochrome family,
249 whereas blue light is absorbed by cryptochrome and phototropin photoreceptors (Lin,
250 2000; Bohne and Linden, 2002). Thus, red and blue lights produce different
251 morphogenetic and photosynthetic responses in plants. In tomato, illumination with
252 blue LED light was effective in fruit yield increase and quality improvement as well
253 as improvement in disease resistance, while illumination with red LED light induced
254 the accumulation of lycopene along with an increase in total carotenoid content (Alba
255 et al., 2000; Schofield and Paliyath, 2005; Liu et al., 2009; Xu et al., 2012). In a
256 previous study, we reported that red LED light was effective in enhancing carotenoid
257 content, especially the content of β -cryptoxanthin, while blue LED light had no
258 significant effect on the carotenoid content in the flavedo of citrus fruit (Ma et al.,
259 2012b).

260 Fresh broccoli is a perishable immature vegetable which deteriorates or senescences
261 rapidly after harvest at ambient temperature. During storage, the appearance of
262 yellowing and the ethylene climacteric peak are direct indexes for senescence in
263 broccoli. Therefore, it is crucial to delay yellowing and suppress ethylene production
264 of broccoli during postharvest storage (Suzuki et al., 2004; Ma et al., 2009, 2012a). It
265 has been reported that UV-B and UV-C treatments effectively delay floret yellowing
266 and chlorophyll degradation by suppressing the activities of chlorophyll-degrading
267 enzymes in broccoli after harvest, indicating that UV-B and UV-C irradiation were
268 useful methods to suppress senescence and maintain quality of broccoli after harvest
269 (Costa et al., 2006; Aiamla-or et al., 2009, 2010). However, in the UV-A treatment the
270 yellowing process was not inhibited in broccoli after harvest (Aiamla-or et al., 2009).

271 In the present study, the results showed that red LED light delayed the yellowing
272 process and decreased ethylene production of broccoli after harvest. In contrast, the
273 yellowing process and ethylene production were not significantly affected by the blue
274 LED light treatment. These results suggested that irradiation with red LED light was
275 effective for delaying senescence in broccoli after harvest.

276 Fresh broccoli, which is rich in AsA, is a good source of AsA intake for humans.
277 However, the content of AsA decreased rapidly to a low level in broccoli after harvest.
278 In the recent years, various research into the improvement of AsA level in broccoli
279 after harvest has been performed (Shigenaga et al., 2005; Mori et al., 2009; Ma et al.,
280 2010, 2012a). Lemoine et al., (2010) reported that the combination of UV-C and hot
281 air can delay the postharvest loss of AsA in broccoli. In the previous study, we found
282 that the applications of 1-MCP and electrostatic atomization were effective methods
283 to suppress AsA loss in broccoli (Ma et al., 2010, 2012a). In the present study, the
284 results showed that the blue LED light treatment did not affect AsA metabolism, while
285 the red LED light treatment was effective in improving the nutritional value of
286 broccoli by delaying AsA reduction after harvest. In addition, AsA as a critical
287 antioxidative component is effective in scavenging ROS, which is closely related with
288 the senescence of fruit and vegetables. In the present study, the higher AsA level in
289 the red LED light treatment indicated that red LED light enhanced the antioxidant
290 capability to scavenge ROS, which might lead to delaying senescence of broccoli
291 after harvest.

292 Although red LED light is effective in delaying senescence and maintaining
293 nutritional value, it is inconvenient for customers to select broccoli in the supermarket
294 under the red light. Thus, in the present study we designed a type of modified white
295 LED light. In this kind of modified white LED light, the ratio of blue light was

296 decreased, while the ratio of red light was increased (Fig. 4). Compared with the
297 control, the reduction of AsA in broccoli after harvest was delayed by the modified
298 white LED light. The AsA content was higher in the modified white LED light
299 treatment than that of the control on the first and second days after harvest. However,
300 under the modified white LED light, the yellowing process and ethylene production
301 were not significantly suppressed, indicating that the senescence process was not
302 delayed by the modified white LED light in broccoli after harvest. Thus, to enhance
303 the efficacy of the modified white LED light, more research is still needed in the
304 future, including (i) further increase in the ratio of the red light in the modified white
305 LED light; (ii) increase in the intensity of modified white LED light; (iii) change the
306 operational mode of the modified white LED light, such as pulsed irradiation.

307 In broccoli, AsA accumulation is controlled by biosynthesis, oxidation and
308 regeneration processes. In the past decade, the genes related to AsA metabolism have
309 been cloned, and their expression profiles have been extensively investigated in
310 broccoli after harvest (Nishikawa et al., 2003; Ma et al., 2012a). Nishikawa et al.,
311 (2003) reported that the regulation of gene expression appeared to be an important
312 mechanism by which AsA metabolism was regulated during the senescence process in
313 broccoli. In the previous study, we found that postharvest suppression of the AsA
314 reduction by 1-MCP and electrostatic atomization was highly regulated at the
315 transcriptional level (Ma et al., 2010, 2012a). In the present study, the results showed
316 that the expression of *BO-VTC2* and *BO-GLDH* was up-regulated on the first and
317 second days, and the expression of *BO-VTC1* was up-regulated on the second day
318 after harvest by the modified white LED light. The up-regulation of *BO-VTC2* and
319 *BO-GLDH*, which are important AsA biosynthetic genes, was consistent with the
320 higher AsA content in broccoli treated by the modified white LED light on the first

321 and second days after harvest.

322 In higher plants, APX is the key enzyme responsible for AsA oxidation. In broccoli,
323 two cytosolic APX genes, *BO-APX1* and *BO-APX2* were identified. Under the
324 modified white LED light, the expression of *BO-APX1* was up-regulated on the first
325 and second day after harvest. The expression of *BO-APX2* was down-regulated on the
326 first day, while it was up-regulated on the second day by the modified white LED
327 light. The expression of *BO-sAPX*, which is a chloroplast APX, was up-regulated on
328 the first day, and was similar to the control on the second day. The regulation of
329 expression of *BO-APX1*, *BO-APX2*, and *BO-sAPX* was not well correlated with the
330 higher AsA content on the first and second days, suggesting that regulation of APX at
331 the transcriptional level might not play a crucial role in suppression of AsA reduction
332 in response to the modified white LED light treatment in broccoli after harvest.
333 Similar results were also observed in broccoli treated by the electrostatic atomization
334 (Ma et al., 2012a).

335 MDAR and DHAR are two enzymes responsible for AsA regeneration in plants
336 (Wheeler et al., 1998; Mittler, 2002; Apel and Hirt, 2004; Sairam and Tyagi, 2004).
337 With the treatment of electrostatic atomization, the up-regulation of the gene
338 expression of *BO-DHAR*, *BO-MDAR1*, and *BO-MDAR2* contributed to the
339 suppression of AsA reduction in broccoli after harvest (Ma et al., 2012a). In the
340 present study, the gene expression of *BO-DHAR* was down-regulated by the modified
341 white LED light, which was not correlated with the higher AsA content. In contrast,
342 the up-regulation of the gene expression of *BO-MDAR1* and *BO-MDAR2* was
343 consistent with the higher AsA content in broccoli treated by the modified white LED
344 light on the first and second days after harvest. Taken together, these results suggested
345 that the suppression of AsA reduction by the modified white LED light treatment was

346 attributed to the up-regulation of the AsA biosynthetic genes (*BO-VTC2* and
347 *BO-GLDH*) and AsA regeneration genes (*BO-MDAR1* and *BO-MDAR2*).

348

349 **5. Conclusion**

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

359 **Acknowledgement**

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

363 **References**

- 364 Aiamla-or, S., Kaewsuksaeng, S., Shigyo, M., Yamauchi, N., 2010. Impact of UV-B
365 irradiation on chlorophyll degradation and chlorophyll-degrading enzyme activities
366 in stored broccoli (*Brassica oleracea* L. Italica Group) florets. Food Chem. 120,
367 645-651.
- 368 Aiamla-or, S., Yamauchi, N., Takino, S., Shigyo, M., 2009. Effect of UV-A and UV-B
369 irradiation on broccoli (*Brassica oleracea* L. Italica Group) floret yellowing during
370 storage. Postharvest Biol. Technol. 54, 177-179.

371 Alba, R., Cordonnier-Pratt, M.M., Pratt, L.H., 2000. Fruit-localized phytochromes
 372 regulate lycopene accumulation independently of ethylene production in tomato.
 373 Plant Physiol. 123, 363-370.

374 Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and
 375 signal transduction. Annu. Rev. Plant Biol. 55, 373-399.

376 Bohne, F., Linden, H., 2002. Regulation of carotenoid biosynthesis genes in response
 377 to light in *Chlamydomonas reinhardtii*. Biochim. Biophys. Acta. 1579, 26-34.

378 Briggs, W.R., Beck, C.F., Cashmore, A.R., Christie, J.M., Hughes, J., Jarillo, J.A.,
 379 Kagawa, T., Kanegae, H., Liscum, E., Nagatani, A., Okada, K., Salomon, M.,
 380 Rudiger, W., Sakai, T., Takano, M., Wada, M., Watson, J.C., 2001. The phototropin
 381 family of photoreceptors. Plant Cell 13, 993-997.

382 Cartea, M.E., Velasco, P., 2008. Glucosinolates in *Brassica* foods: Bioavailability in
 383 food and significance for human health. Phytochem. Rev. 7, 213-229.

384 Costa, L., Vicente, A.R., Civello, P.M., Chaves, A.R., Martínez, G.A., 2006. UV-C
 385 treatment delays postharvest senescence in broccoli florets. Postharvest Biol.
 386 Technol. 39, 204-210.

387 Downs, C.G., Somerfield, S.D., Davey, M.C., 1997. Cytokinin treatment delays
 388 senescence but not sucrose loss in harvested broccoli. Postharvest Biol. Technol. 11,
 389 93-100.

390 Fernández-León, M.F., Fernández-León, A.M., Lozano, M., Ayuso, M.C.,
 391 González-Gómez, D., 2013. Altered commercial controlled atmosphere storage
 392 conditions for 'Parhenon' broccoli plants (*Brassica oleracea* L. var. *italica*).
 393 Influence on the outer quality parameters and on the health-promoting compounds.
 394 LWT - Food Sci. Technol. 50, 665-672.

395 Hansen, M.E., Sørensen, H., Cantwell, M., 2001. Changes in acetaldehyde, ethanol

396 and amino acid concentrations in broccoli florets during air and controlled
 397 atmosphere storage. *Postharvest Biol. Technol.* 22, 227-237.

398 Kato, M., Hayakawa, Y., Hyodo, H., Ikoma, Y., Yano, M., 2000. Wound-induced
 399 ethylene synthesis and expression and formation of
 400 1-aminocyclopropane-1-carboxylate (ACC) synthase, ACC oxidase, phenylalanine
 401 ammonia-lyase, and peroxidase in wounded mesocarp tissue of *Cucurbita maxima*.
 402 *Plant Cell Physiol.* 41, 440-447.

403 Lemoine, M.L., Chaves, A.R., Martínez, G.A., 2010. Influence of combined hot air
 404 and UV-C treatment on the antioxidant system of minimally processed broccoli
 405 (*Brassica oleracea* L.var. *Italica*). *LWT-Food Sci. Technol.* 43,1313-1319.

406 Lin, C., 2000. Plant blue-light receptors. *Trends Plant Sci.* 5, 337-342.

407 Lin, K.H., Huang, M.Y., Huang, W.D., Hsu, M.H., Yang, Z.W., Yang, C.M., 2013. The
 408 effects of red, blue, and white light-emitting diodes on the growth, development,
 409 and edible quality of hydroponically grown lettuce (*Lactuca sativa* L. var. *capitata*).
 410 *Sci. Hortic.* 150, 86-91.

411 Liu, L.H., Zabarar, D., Bennett, L.E., Aguas, P., Woonton, B.W., 2009. Effects of
 412 UV-C, red light and sun light on the carotenoid content and physical qualities of
 413 tomatoes during post-harvest storage. *Food Chem.* 115, 495-500.

414 Ma, G., Wang, R., Wang, C.R., Kato, M., Yamawaki, K., Qin, F.F., Xu, H.L., 2009.
 415 Effect of 1-methylcyclopropene on expression of genes for ethylene biosynthesis
 416 enzymes and ethylene receptors in post-harvest broccoli. *Plant Growth Regul.* 57,
 417 223-232.

418 Ma, G., Zhang, L.C., Kato, M., Yamawaki, K., Asai, T., Nishikawa, F., Ikoma, Y.,
 419 Matsumoto, H., 2010. Effect of 1-methylcyclopropene on the expression of genes
 420 for ascorbate metabolism in postharvest broccoli. *Postharvest Biol. Technol.* 58,

421 121-128.

422 Ma, G., Zhang, L.C., Kato, M., Yamawaki, K., Asai, T., Nishikawa, F., Ikoma, Y.,
 423 Matsumoto, H., Yamauchi, T., Kamisako, T., 2012a. Effect of electrostatic
 424 atomization on ascorbate metabolism in postharvest broccoli. *Postharvest Biol.*
 425 *Technol.* 74, 19-25.

426 Ma, G., Zhang, L.C., Kato, M., Yamawaki, K., Kiriwa, Y., Yahata, M., Ikoma, Y.,
 427 Matsumoto, H., 2012b. Effect of blue and red LED light irradiation on
 428 β -cryptoxanthin accumulation in the flavedo of citrus fruits. *J. Agric. Food Chem.*
 429 60, 197-201.

430 Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*
 431 7, 405-410.

432 Mori, T., H. Terai, N. Yamauchi and Y. Suzuki., 2009. Effects of postharvest ethanol
 433 vapor treatment on the ascorbate-glutathione cycle in broccoli florets. *Postharvest*
 434 *Biol. Technol.* 52, 134-136.

435 Nishikawa, F., Kato, M., Hyodo, H., Ikoma, Y., Sugiura, M., Yano, M., 2003.
 436 Ascorbate metabolism in harvested broccoli. *J. Exp. Bot.* 54, 2439-2448.

437 Podsdek, A., 2007. Natural antioxidants and antioxidant capacity of *Brassica*
 438 vegetables: A review. *LWT - Food Sci. and Technol.* 40, 1-11.

439 Rushing, J.W., 1990. Cytokinins affect respiration, ethylene production, and
 440 chlorophyll retention of packaged broccoli florets. *HortScience* 25, 88-90.

441 Saebo, A., Krekling, T., Appelgren, M., 1995. Light quality affects photosynthesis and
 442 leaf anatomy of birch plantlets in vitro. *Plant Cell Tissue Organ Culture* 41,
 443 177-185.

444 Sairam, R.K., Tyagi, A., 2004. Physiology and molecular biology of salinity stress
 445 tolerance in plants. *Curr. Sci.* 86, 407-421.

446 Schofield, A., Paliyath, G., 2005. Modulation of carotenoid biosynthesis during
 447 tomato fruit ripening through phytochrome regulation of phytoene synthase activity.
 448 Plant Physiol. Biochem. 43, 1052-1060.

449 Shigenaga, T., Yamauchi, N., Funamoto, Y., Shigyo, M., 2005. Effects of heat
 450 treatment on an ascorbate-glutathione cycle in stored broccoli (*Brassica oleracea*
 451 L.) florets. Postharvest Biol. Technol. 38, 152-159.

452 Suzuki, Y., Uji, T., Terai, H., 2004. Inhibition of senescence in broccoli florets with
 453 ethanol vapor from alcohol powder. Postharvest Biol. Technol. 31, 177-182.

454 Toivonen, P.M.A., DeEll, J.R., 2001. Chlorophyll fluorescence, fermentation product
 455 accumulation, and quality of stored broccoli in modified atmosphere packages and
 456 subsequent air storage. Postharvest Biol. Technol. 23, 61-69.

457 Wang, C.Y., 1977. Effect of aminoethoxy analog of rhizobitoxine and sodium
 458 benzoate on senescence of broccoli. HortScience 12, 54-56.

459 Wheeler, G.L., Jones, M.A., Smirnoff, N., 1998. The biosynthetic pathway of vitamin
 460 C in higher plants. Nature 393, 365-369.

461 Wu, M.C., Hou, C.Y., Jiang, C.M., Wang, Y.T., Wang, C.Y., Chen, H.H., Chang, H.M.,
 462 2007. A novel approach of LED light radiation improves the antioxidant activity of
 463 pea seedlings. Food Chem. 101, 1753-1758.

464 Xu, H.L., Xu, Q.C., Li, F.L., Feng, Y.Z., Qin, F.F., Fang W., 2012. Applications of
 465 xerophytophysiology in plant production—LED blue light as a stimulus improved
 466 the tomato crop. Sci. Hort. 148, 190-196.

467 Zhan, L.J., Hu, J.Q., Li, Y., Pang, L.G., 2012. Combination of light exposure and low
 468 temperature in preserving quality and extending shelf-life of fresh-cut broccoli
 469 (*Brassica oleracea* L.) Postharvest Biol. Technol. 72, 76-81.

Susanne Baldermann

Technische Universität Braunschweig, Institute of Food Chemistry, Schleinitzstrasse
20, 38106 Braunschweig, Germany

E-mail: s_baldermann@yahoo.de

Yasutaka Kubo

Laboratory of Postharvest Agriculture, Faculty of Agriculture, Okayama University,
Tsushima, Okayama 700-8530, Japan

Tel.: +81 86 251 8338

Fax: +81 86 251 8338

E-mail: ykubo@cc.okayama-u.ac.jp

Yasuo Suzuki

Graduate School of Agricultural Science, Kobe University, Rokkodai-cho 1-1,
Nada-ku, Kobe 657-8501, Japan

Tel.: +81 78 803 5834;

Fax: +81 78 803 5834.

E-mail: ysuzuki@kobe-u.ac.jp

Naoki Yamauchi

Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515,
Japan

Tel.: +81-83-933-5843

Fax: +81-83-933-5843

E-mail: yamauchi@agr.yamaguchi-u.ac.jp

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 $\pm$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Control

Red

Blue