

Effect of the combination of ethylene and red LED light irradiation on carotenoid accumulation and carotenogenic gene expression in the flavedo of citrus fruit

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1 **Running title: Effect of ethylene and red LED lights on carotenoid metabolism in citrus**
2 **Effect of the combination of ethylene and red LED light irradiation on**
3 **carotenoid accumulation and carotenogenic gene expression in the flavedo of**
4 **citrus fruit**

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19 **ABSTRACT**

20 In the present study, the effects of ethylene and red light-emitting diode (LED) light
21 (660 nm) on the accumulation of carotenoids and expression of genes related to
22 carotenoid biosynthesis were investigated in the flavedo of Satsuma mandarin. The
23 results showed that the contents of β -cryptoxanthin, all-*trans*-violaxanthin,
24 9-*cis*-violaxanthin and lutein were simultaneously increased along with the total
25 carotenoid accumulation by the red LED light. With the ethylene treatment, the
26 contents of β -carotene and β -cryptoxanthin were increased, while the content of lutein
27 was decreased in the flavedo of Satsuma mandarin. The suppression of lutein
28 accumulation by ethylene was inhibited when the ethylene treatment was performed
29 under the red LED light. With the combination of ethylene and red LED light
30 treatments, the contents of β -cryptoxanthin and lutein were simultaneously increased.
31 Gene expression results showed that simultaneous increases in the expression of
32 *CitPSY*, *CitPDS*, *CitZDS*, *CitCRTISO*, *CitLCYb1*, *CitLCYb2*, *CitLCYe*, *CitHYb*, and
33 *CitZEP* contributed to the accumulation of β -cryptoxanthin and lutein in the treatment
34 of ethylene combined with red LED light. The results presented herein might provide
35 new strategies to enhance the commercial and nutritional value of citrus fruit.

36

37 **KEYWORDS:** Citrus; β -cryptoxanthin; flavedo; lutein; red LED light

38

39 **1 Introduction**

40 Carotenoids, important natural isoprenoid pigments, fulfill a variety of important
41 functions in plants and play a critical role in human nutrition and health (Schwartz et
42 al., 1997; Cunningham and Gantt, 1998; Havaux, 1998; Krinsky et al., 2003; Ledford
43 and Niyogi, 2005). In citrus fruit, carotenoids are responsible for the external and
44 internal coloration, and their contents and compositions are important indexes for the
45 commercial and nutritional quality of the fruit. The accumulation of carotenoids in
46 citrus fruit has been extensively investigated over the past decade (Kato et al., 2004;
47 Rodrigo et al., 2004; Kato et al., 2006; Rodrigo and Zacarías, 2007; Kato, 2012;
48 Zhang et al., 2012a; Ma et al., 2013). Moreover, genes encoding enzymes for the main
49 steps of carotenoid biosynthesis have been isolated and their expression was
50 characterized in the flavedo and juice sacs of different citrus varieties (Kato et al.,
51 2004; 2007; Alquézar et al., 2008; Alquézar et al., 2009; Fig. 1). In the previous
52 studies, we found that as fruit maturation progressed, a simultaneous increase in the
53 expression of genes (*CitPSY*, *CitPDS*, *CitZDS*, *CitLCYb*, *CitHYb*, and *CitZEP*) led to
54 massive β,β -xanthophyll accumulation in the flavedo and juice sacs of Satsuma
55 mandarin and Valencia orange (Kato et al., 2004). In addition, the cyclization of
56 lycopene by *CitLCYb1* and *CitLCYb2* played an important role in determining the
57 profiles of carotenoids in the orange stage of the citrus fruit (Zhang et al., 2012b)

58 Light is an important environmental factor for plants. It is not only an essential
59 energy source for plants, but also an important signal for plants growth and
60 development (Chory et al., 1996; Clouse, 2001; Kim et al., 2002). In higher plants,

61 sensing of light is carried out by various light photoreceptors (Briggs et al., 2001).
62 Thus, plants exhibit different responses to different wavelengths of lights (Goins et al.,
63 1997; Xu et al., 2011; Jung et al., 2013). Wu et al. (2007) reported that β -carotene
64 content was much higher in the red light-treated group than blue light-treated group in
65 leaves and stems of pea seedlings. In tomatoes, the accumulation of lycopene along
66 with an increase in total carotenoid content was also observed in response to red light
67 treatment (Alba et al., 2000; Schofield and Paliyath, 2005; Liu et al., 2009). In citrus
68 fruit, the red LED light was effective to enhance carotenoids contents, especially the
69 content of β -cryptoxanthin, while blue LED light had no significant effect on the
70 carotenoids contents in the flavedo of Satsuma mandarin (Ma et al., 2012). Even
71 though citrus fruit are non-climacteric and produce a low level of ethylene during
72 ripening, they are sensitive to exogenous ethylene. In the recent year, exogenous
73 application of ethylene has been widely employed to enhance the external coloration
74 of citrus fruit. Rodrigo and Zacarías (2007) reported that exogenous ethylene
75 treatment increased the contents of carotenoids; as a result, the degreening process of
76 citrus fruit was accelerated. To date, however, information on the effects of the
77 combination of ethylene and the red LED light on carotenoid accumulation in citrus is
78 still unknown. As citrus fruit pulp matures earlier than the peel, the pulp reaches
79 maturity and is edible, while the peel is still green in October. In the present study, to
80 promote peel degreening and improve the carotenoids contents and compositions in
81 the flavedo of citrus fruit, the effects of red LED light (660nm) and ethylene on
82 carotenoid accumulation and the expression of genes related to carotenoid

83 biosynthesis were investigated. The results presented herein might provide new
84 strategies to enhance the carotenoid production in citrus fruit.

85 **2 Materials and methods**

86 *2.1 Plant Materials*

87 Fruit of Satsuma mandarin (*Citrus unshiu* Marc.) were harvested in October 150
88 day after anthesis at the Fujieda Farm of Shizuoka University (Shizuoka, Japan). In
89 this stage, the fruit peel just begins to degreen with the accumulation of carotenoids.
90 Fruit 45-50 mm in diameter and light green in color were used as materials.

91 *2.2 Treatment*

92 Fruit were placed in 35-L sealed plastic chambers. The fruit were continuously
93 treated for 6 days at 20 °C as follows: with 150 $\mu\text{mol m}^{-2}\text{s}^{-1}$ red (660 nm) LED lights;
94 with 50 $\mu\text{L L}^{-1}$ ethylene in the dark; with 50 $\mu\text{L L}^{-1}$ of ethylene under 150 $\mu\text{mol m}^{-2}\text{s}^{-1}$
95 red LED lights. Fruit stored at 20 °C in the dark were used as the control. After each
96 treatment, the flavedo was immediately frozen in liquid nitrogen, and kept at – 80 °C
97 until used.

98 *2.3 Color measurement*

99 Color measurement was carried out with a colorimeter (NR-11, Nippon Denshoku,
100 Japan). The CIE 1976 $L^*a^*b^*$ color scale was adopted. The hue angle [$H^\circ = \arctangent$
101 (b^*/a^*) and Citrus color index (CCI) = $[1000 \times a^* / (L^* \times b^*)]$ were calculated according
102 to methods previously reported (Zhou et al., 2010).

103 *2.4 Extraction and determination of carotenoids*

104 The identification, extraction and quantification of carotenoid in citrus have been

105 described previously (Kato et al., 2004). β -Carotene, β -cryptoxanthin,
106 all-*trans*-violaxanthin, 9-*cis*-violaxanthin and lutein were quantified in the flavedo of
107 Satsuma mandarin during the experimental period. The contents of carotenoids were
108 expressed as $\mu\text{g g}^{-1}$ fresh weight. Carotenoid quantification was performed in three
109 replicates.

110 2.4 Total RNA extraction and real-time quantitative RT-PCR

111 Total RNA was extracted from the flavedo of Satsuma mandarin fruit according to a
112 previously reported method (Kato et al., 2004). The total RNA was cleaned up with
113 the RNeasy Mini Kit (Qiagen, Hilden, Germany) with on-column DNase digestion.
114 The reactions of reverse transcription (RT) were performed with 2 μg of purified RNA
115 and a random hexamer at 37 °C for 60 min using TaqMan Reverse Transcription
116 Reagents (Applied Biosystems).

117 TaqMan MGB probes and sets of primers for *CitPSY*, *CitPDS*, *CitZDS*, *CitCRTISO*,
118 *CitLCYb1*, *CitLCYb2*, *CitLCYe*, *CitHYb*, *CitZEP* and *CitVDE* were designed
119 according to Ma et al. (2013; Table 1). For endogenous control, the TaqMan
120 Ribosomal RNA Control Reagents VIC Probe (Applied Biosystems) was used.
121 TaqMan real-time PCR was carried out with the TaqMan Universal PCR Master Mix
122 (Applied Biosystems) using StepOnePlus™ Real-Time PCR System (Applied
123 Biosystems) according to the manufacture's instructions. Each reaction mixture
124 contained 900 nM primers, a 250 nM TaqMan MGB Probe, and template cDNA. The
125 thermal cycling conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for
126 15 s and 60 °C for 60 s. The levels of gene expression were analyzed with

127 StepOnePlus™ Real-Time PCR System Software (Applied Biosystems) and
128 normalized with the results of 18S ribosomal RNA. Real-time quantitative RT-PCR
129 was performed in three replicates for each sample.

130 *2.5 Statistical analysis*

131 All values are shown as the means \pm SE for three replicates. The data were
132 analyzed, and Tukey's HSD test was used to compare the means at $P < 0.05$.

133

134 **3 Results and discussion**

135 *3.1 Effects of red LED light and ethylene on fruit color*

136 In the present study, the changes in the color were described by the hue angle (H°)
137 and CCI. H° value ranges from 0° to 360° with 0° for red-purple, 90° for yellow, and
138 180° for bluish green. For CCI, positive values are for red, negative values are for
139 blue-green, and 0 is for an intermediate mixture of red, yellow, and blue-green. In the
140 control, the H° decreased while the CCI increased during the storage, indicating that
141 the color of flavedo turned yellow gradually. Both ethylene and red LED light
142 treatments can accelerate the changes in H° and CCI in flavedo of Satsuma mandarin
143 during the storage. In the combination of ethylene and red LED light treatments, the
144 changes in H° and CCI were more significant than ethylene or red LED light
145 treatment alone, and the color of flavedo turned deeper yellow color on the sixth day
146 (Fig. 2 A and B). In addition, as shown in the Fig. 2 C, the peel turned completely
147 yellow in the ethylene treatment on the sixth day after harvest. In the treatment of red
148 LED light, non-uniform color with light green were observed in the peel on the sixth

149 day after harvest. These result suggested that ethylene is more effective to induce
150 chlorophyll breakdown than the red LED irradiation, which led to a better color in the
151 ethylene treatment on the six day after harvest.

152 *3.2 Effects of red LED light and ethylene on carotenoid content and composition*

153 We previously reported that irradiation with red light at intensity of $50 \mu\text{mol m}^{-2}\text{s}^{-1}$
154 for six days was effective to enhance carotenoids contents, especially the content of
155 β -cryptoxanthin; while blue LED light had no significant effect on the carotenoid
156 content in the flavedo of Satsuma mandarin (Ma et al., 2012). In the present study, we
157 increased the intensity of the red LED light to $150 \mu\text{mol m}^{-2}\text{s}^{-1}$, and the results showed
158 that the contents of β -cryptoxanthin, all-*trans*-violaxanthin, 9-*cis*-violaxanthin and
159 lutein were simultaneously increased along with the total carotenoid accumulation
160 under red LED light. With the ethylene treatment, the contents of β -carotene and
161 β -cryptoxanthin were increased, while the contents of lutein, all-*trans*-violaxanthin,
162 and 9-*cis*-violaxanthin were decreased. The total carotenoid content was not
163 significantly affected by the ethylene treatment. In contrast, when the ethylene
164 treatment was performed under red LED light, the contents of β -carotene,
165 β -cryptoxanthin, all-*trans*-violaxanthin and lutein were increased, and the total
166 carotenoid content was higher than that in the ethylene treatment alone (Fig. 3).

167 In the present study, the results showed that ethylene exhibited different effects on
168 the accumulation of individual carotenoids. The ethylene treatment decreased the
169 content of lutein, which is the major β,ϵ -carotenoid accumulating in the green stage.
170 While it increased the contents of β -carotene and β -cryptoxanthin in the flavedo of

171 Satsuma mandarin. In citrus fruit, the carotenoid biosynthetic pathway changes from
172 β,ϵ -carotenoid synthesis to β,β -carotenoid synthesis during the ripening process (Kato
173 et al., 2004; Zhang et al., 2012b). In this study, the changes of the carotenoids
174 compositions in the flavedo of ethylene-treated fruit resembled those ripening on tree.
175 These results suggested that ethylene was involved in the regulation of carotenoid
176 accumulation in citrus fruit during the ripening process. The similar results were also
177 observed in Navelate oranges (Rodrigo and Zacarías, 200). Matsumoto et al. (2009)
178 reported that ethylene treatment increased the contents of carotenes and
179 β -cryptoxanthin, and as a result the total carotenoid content was increased
180 dramatically in the flavedo of Satsuma mandarin harvested in the orange stage.
181 However, the results presented herein showed that the ethylene treatment did not
182 increase the total carotenoid content in the flavedo of Satsuma mandarin harvested in
183 the green stage because of the rapid decrease of lutein. Thus, the effects of ethylene
184 on the total carotenoid accumulation in citrus fruit were distinct in the different
185 ripening stages.

186 In addition, we found that the suppression effect of ethylene on lutein accumulation
187 in the flavedo of the green stage could be inhibited by red LED light irradiation. The
188 contents of lutein, β -carotene, β -cryptoxanthin, and all-*trans*-violaxanthin were
189 simultaneously increased when the ethylene treatment was performed under red LED
190 light. In general, consumers eat the pulp of citrus fruits. In the recent years, however,
191 citrus peel becomes more popular in human diet, for example it is added fresh as zest,
192 or dried and candied for use in food and drinks. It has been proven that consuming the

193 citrus peel is beneficial to human health because of its high levels of vitamins,
194 minerals, antioxidants and carotenoids. In Satsuma mandarin, the contents of
195 carotenoids and antioxidants are much higher in the peel than the pulp (Kato et al.,
196 2004). In the present study, the results showed that the contents of β -cryptoxanthin
197 and lutein, which are important antioxidants beneficial to human health, were
198 increased by the combination treatments of ethylene and red LED light. These results
199 suggested that the postharvest application of ethylene combined with the red LED
200 light irradiation contributed to improve the nutritional value of citrus fruit.

201 *3.2 Effects of red LED light and ethylene on gene expression related to carotenoid* 202 *metabolism*

203 The transcriptional regulation of carotenoid biosynthetic genes is a major
204 mechanism by which the accumulations of specific carotenoids are regulated in the
205 flavedo and juice sacs (Kato et al., 2004, 2006; Ma et al., 2012; Zhang et al., 2012).
206 As shown in Fig. 4, the expression of *CitPSY*, *CitCRTISO*, *CitLCYb2*, *CitLCYe*, and
207 *CitVDE* was up-regulated by the red LED light treatment. With the ethylene treatment,
208 the expression of a set of genes related to β,β -xanthophylls biosynthesis (*CitPSY*,
209 *CitPDS*, *CitZDS*, *CitCRTISO*, *CitLCYb1*, *CitLCYb2*, *CitHYb*, and *CitZEP*) was
210 simultaneously up-regulated. In contrast, the expression of *CitLCYe* was
211 down-regulated by ethylene. Under red LED light, the expression of *CitPSY*, *CitPDS*,
212 *CitZDS*, *CitCRTISO*, *CitLCYb1*, *CitLCYb2*, *CitLCYe*, *CitHYb*, and *CitZEP* was
213 significantly up-regulated by the ethylene treatment, which contributed to the
214 accumulation of β -cryptoxanthin and lutein. Moreover, the expression levels of

215 *CitPSY*, *CitPDS*, *CitZDS*, *CitLCYb1*, *CitLCYb2*, *CitLCYe*, and *CitHYb* were higher
216 than those treated by ethylene alone.

217 In citrus fruit, the drastically decrease in the expression of *CitLCYe* was responsible
218 for the decrease of lutein, and accelerated the pathway from β,ϵ -carotenoid synthesis
219 to β,β -carotenoid synthesis during the ripening process (Kato et al., 2004; Zhang et al.,
220 2012b). In the present study, the results showed that the ethylene treatment
221 down-regulated expression of *CitLCYe*, which led to the decrease of lutein. However,
222 when the ethylene treatment was performed under red LED light, the expression of
223 *CitLCYe* was significantly increased, and the simultaneous increases in the expression
224 of *CitPSY*, *CitPDS*, *CitZDS*, *CitLCYb1*, *CitLCYb2*, *CitLCYe*, *CitHYb*, and *CitZEP*
225 contributed to the accumulation of β -cryptoxanthin and lutein in the ethylene
226 treatment under the red LED light.

227 **4. Conclusion**

228 In the present study, the effects of combination of red LED light and ethylene on
229 carotenoid accumulation in the flavedo of citrus fruit have been studied. The results
230 showed that the combination of ethylene treatment and the red LED light was
231 effective to increase the contents of β -cryptoxanthin and lutein in the flavedo of citrus
232 fruit. Moreover, simultaneous increases in the expression of *CitPSY*, *CitPDS*, *CitZDS*,
233 *CitCRTISO*, *CitLCYb1*, *CitLCYb2*, *CitLCYe*, *CitHYb*, and *CitZEP* contributed to the
234 accumulation of β -cryptoxanthin and lutein in the ethylene treatment under the red
235 LED light. These results indicated that the combination of ethylene and red LED light
236 treatment was effective to enhance the commercial and nutritional value of citrus fruit.

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241

242 **References**

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

246 Alquézar, B., Rodrigo, M.J., Zacarías, L., 2008. Regulation of carotenoid biosynthesis
247 during fruit maturation in the red-fleshed orange mutant Cara Cara. *Phytochemistry*
248 69, 1997-2007.

249 Alquézar, B., Zacarías, L., Rodrigo, M.J., 2009. Molecular and functional
250 characterization of a novel chromoplast-specific lycopene β -cyclase from *Citrus*
251 and its relation to lycopene accumulation. *J. Exp. Bot.* 60, 1783-1797.

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

256 Chory, J., Chatterjee, M., Cook, R.K., Elich, T., Fankhauser, C., Li, J., Nagpal, P.,
257 Neff, M., Pepper, A., Poole, D., Reed, J., Vitart, V., 1996. From seed germination to
258 flowering, light controls plant development via the pigment phytochrome. *Proc.*

259 Natl. Acad. Sci. USA 93, 12066-12071.

260 Clouse, S.D., 2001. Integration of light and brassinosteroid signals in etiolated
261 seedling growth. *Trends in Plant Sci.* 6, 443-445.

262 Cunningham, F.X., Gantt, E., 1998. Genes and enzymes of carotenoid biosynthesis in
263 plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 557-583.

264 Goins, G.D., Yorio, N.C., Sanwo, M.M., Brown, C.S., 1997. Photomorphogenesis,
265 photosynthesis, and seed yield of wheat plants grown under red light-emitting
266 diodes (LEDs) with and without supplemental blue lighting. *J. Exp. Bot.* 48,
267 1407-1413.

268 Havaux, M., 1998. Carotenoids as membrane stabilizers in chloroplasts. *Trends Plant*
269 *Sci.* 3, 147-151.

270 Jung, E.S., Lee, S., Lim, S.H., Ha, S.H., Liu, K.H., Lee, C.H., 2013. Metabolite
271 profiling of the short-term responses of rice leaves (*Oryza sativa* cv. Ilmi) cultivated
272 under different LED lights and its correlations with antioxidant activities. *Plant Sci.*
273 210, 61-69.

274 Kato, M., 2012. Mechanism of carotenoid accumulation in citrus fruit. *J. Jpn. Soc.*
275 *Hortic. Sci.* 81, 219-233.

276 Kato, M., Ikoma, Y., Matsumoto, H., Sugiura, M., Hyodo, H., Yano, M., 2004.
277 Accumulation of carotenoids and expression of carotenoid biosynthetic genes
278 during maturation in citrus fruit. *Plant Physiol.* 134, 824-837.

279 Kato, M., Matsumoto, H., Ikoma, Y., Kuniga, T., Nakajima, N., Yoshida, T., Yano, M.,
280 2007. Accumulation of carotenoids and expression of carotenoid biosynthetic genes

281 and carotenoid cleavage dioxygenase genes during fruit maturation in the juice sacs
282 of 'Tamami,' 'Kiyomi' tangor, and 'Wilking' mandarin. *J. Jpn. Soc. for Hortic. Sci.*
283 76, 103-111.

284 Kato, M., Matsumoto, H., Ikoma, Y., Okuda, H., Yano, M., 2006. The role of
285 carotenoid cleavage dioxygenases in the regulation of carotenoid profiles during
286 maturation in citrus fruit. *J. Exp. Bot.* 57, 2153-2164.

287 Kim, T.H., Kim, B.H., von Arnim, A.G., 2002. Repressors of photomorphogenesis. *Int.*
288 *Rev. Cytol.* 220, 185-223.

289 Ledford, H.K., Niyogi, K.K., 2005. Singlet oxygen and photo-oxidative stress
290 management in plants and algae. *Plant Cell Environ.* 28, 1037-1045.

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

294 Ma G, Zhang, L.C., Matsuta, A., Matsutani, K., Yamawaki, K., Yahata, M., Wahyudi,
295 A., Motohashi, R., Kato, M., 2013. Enzymatic formation of β -citraurin from
296 β -cryptoxanthin and zeaxanthin by carotenoid cleavage dioxygenase4 in the
297 flavedo of citrus fruit. *Plant Physiol.* 163, 682-695.

298 Ma, G., Zhang, L.C., Kato M., Yamawaki, K., Kiriwa, Y., Yahata, M., Ikoma, Y.,
299 Matsumoto, H., 2012. Effect of blue and red LED light irradiation on
300 β -cryptoxanthin accumulation in the flavedo of citrus fruits. *J. Agric. Food Chem.*
301 60, 197-201.

302 Matsumoto, A., Mizukami, H., Mizuno, S., Umegaki, K., Nishikawa, J., Shudo, K.,

303 Kagechika, H., Inoue, M., 2007. β -Cryptoxanthin, a novel natural RAR ligand,
304 induces ATP-binding cassette transporters in macrophages. *Biochem. Pharmacol.*
305 74, 256-264.

306 Matsumoto, H., Ikoma, Y., Kato, M., Nakajima, N., Hasegawa, Y., 2009. Effect of
307 postharvest temperature and ethylene on carotenoid accumulation in the flavedo
308 and juice sacs of Satsuma mandarin (*Citrus unshiu* Marc.) fruit. *J. Agric. Food*
309 *Chem.* 57, 4724-4732.

310 Rodrigo, M.J., Marcos, J.F., Zacarías, L., 2004. Biochemical and molecular analysis of
311 carotenoid biosynthesis in flavedo of orange (*Citrus sinensis* L.) during fruit
312 development and maturation. *J. Agric. Food Chem.* 52, 6724-6731.

313 Rodrigo, M.J., Zacarías, L., 2007. Effect of postharvest ethylene treatment on
314 carotenoid accumulation and the expression of carotenoid biosynthetic genes in the
315 flavedo of orange (*Citrus sinensis* L. Osbeck) fruit. *Postharvest Biol. Technol.* 43,
316 14-22.

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

320 Schwartz, S.H., Tan, B.C., Gage, D.A., Zeevaart, J.A., McCarty, D.R., 1997. Specific
321 oxidative cleavage of carotenoids by VP14 of maize. *Science* 276, 1872-1874.

322 Wu, M.C., Hou, C.Y., Jiang, C.M., Wang, Y.T., Wang, C.Y., Chen, H.H., Chang, H.M.,
323 2007. A novel approach of LED light radiation improves the antioxidant activity of
324 pea seedlings. *Food Chem.* 101, 1753-1758.

325 Xu, J., Liu, B.Z., Liu, X., Gao, H.J., Deng, X.X., 2011. Carotenoids synthesized in
326 citrus callus of different genotypes. *Acta Physiol. Plant* 33, 745-753.

327 Zhang, L.C., Ma, G., Kato M., Yamawaki, K., Takagi, T., Kiriwa, Y., Ikoma, Y.,
328 Matsumoto, H., Yoshioka, T., Nesumi, H., 2012a. Regulation of carotenoid
329 accumulation and the expression of carotenoid metabolic genes in citrus juice sacs
330 *in vitro*. *J. Exp. Bot.* 63, 871-886.

331 Zhang, L.C., Ma, G., Shirai, Y., Kato, M., Yamawaki, K., Ikoma, Y., Matsumoto, H.,
332 2012b. Expression and functional analysis of two lycopene β -cyclases from citrus
333 fruits. *Planta* 236, 1315-1325.

334 Zhou, J.L., Sun, C.D., Zhang, L.L., Dai, X., Xu, C.J., Chen, K.S., 2010. Preferential
335 accumulation of orange-colored carotenoids in Ponkan (*Citrus reticulata*) fruit peel
336 following postharvest application of ethylene or ethephon. *Scientia Horticulturae* 126,
337 229-235.

FIGURE LEGENDS

Fig. 1. Carotenoid biosynthetic pathway in citrus. GGPP, geranylgeranyl diphosphate. Enzymes are named according to the designation of their genes. PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ -carotene desaturase; CRTISO, carotenoid isomerase; LCYb, lycopene β -cyclase; LCYe, lycopene ϵ -cyclase; HYe, ϵ -ring hydroxylase; HYb, β -ring hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase.

Fig. 2. Effect of red LED light and ethylene on fruit color during the storage. A, H° . B, CCI. C, The photos of the citrus fruit on the sixth day after harvest. $H^{\circ} = \arctangent(b^*/a^*)$; Citrus color index (CCI) = $[1000 \times a^*/(L^* \times b^*)]$.

Fig. 3. Effect of red LED light and ethylene on the carotenoid content in the flavedo of citrus fruit. β -Car, β -carotene. β -Cry, β -cryptoxanthin. T-vio, all-trans-violaxanthin. C-vio, 9-cis-violaxanthin. Lut, lutein. Total car, Total carotenoid. C, control. R, red. E, ethylene. R+E, combination treatment with red and ethylene. The value for total carotenoid was the sum of identified carotenoids. Columns and bars represent the means and SE (n=3), respectively. Different letters indicate significant differences at the 5% level by Tukey's HSD test.

Fig. 4. Effect of red LED light and ethylene on the expression of carotenoid metabolism related genes in the flavedo of citrus fruit. C, control. R, red. E, ethylene. R+E, combination treatment with red and ethylene. 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. Columns and bars represent the means and SE (n=3), respectively.

Different letters indicate significant differences at the 5% level by Tukey's HSD test.

Table 1 Primer sequences and TaqMan MGB Probes used for the quantitative RT-PCRs of the genes related to carotenoid metabolism

cDNA	Primer sequence	TaqMan MGB Probe
<i>CitPSY</i>	Sense: CGTTGATGGGCCTAATGCTT Antisense :ACCTGGACTCCCACCTGTCTAA	ACACATAACTCCAACAGC
<i>CitPDS</i>	Sense: TGGCAACCCCCAGAGA Antisense: CACCCAGTGACTGAATGTGTT	ACTTTGCTTGCCTATTGT
<i>CitZDS</i>	Sense: AAAGGCACTTGTTGATCCTGATG Antisense: ACCAATCAGAGAAGCTTATACTATCCA	CCTTGAAGGACATACGAGAT
<i>CitCRTISO</i>	Sense: AAAGACACACCGGCGGTATC Antisense: CGAGGCATTGGCCCATAG	AGCTCGCGATCAGG
<i>CitLCYb1</i>	Sense: TGGTACCGCTGGGATGGT Antisense: CAATAGGAGCCGCAGCTAAAGT	CACCCTTCAACTGGCT
<i>CitLCYb2</i>	Sense: CCTTGGCTCAACCAGGATGA Antisense: ACCCATTCACACTTTCTGATGA	CAGAGGCAGGCCAC
<i>CitLCYe</i>	Sense: AAGGTGTGTCGAGTCAGGTGTTT Antisense: CCACTGGTAGATTCCGTAATGCT	ATATCTTAGCTCAAAAGTGG
<i>CitHYb</i>	Sense: GCGGCTCACCAGCTTCAC Antisense: CCGAGAAAGAGCCCATATGG	ACTCGGATAAATTCC
<i>CitZEP</i>	Sense: CTAAAGAGCTATGAGAGAGCTAGGAGACT Antisense: CACTGCGGCCGATCTTG	CGAGTGGCTGTTATC
<i>CitVDE</i>	Sense: CAAAGACTTCAATGGGAAGTGGTA Antisense: TGGCAATCAAAGTATCGAAGGA	TTTCTAGTGGTTTAAATCC

Fig. 1

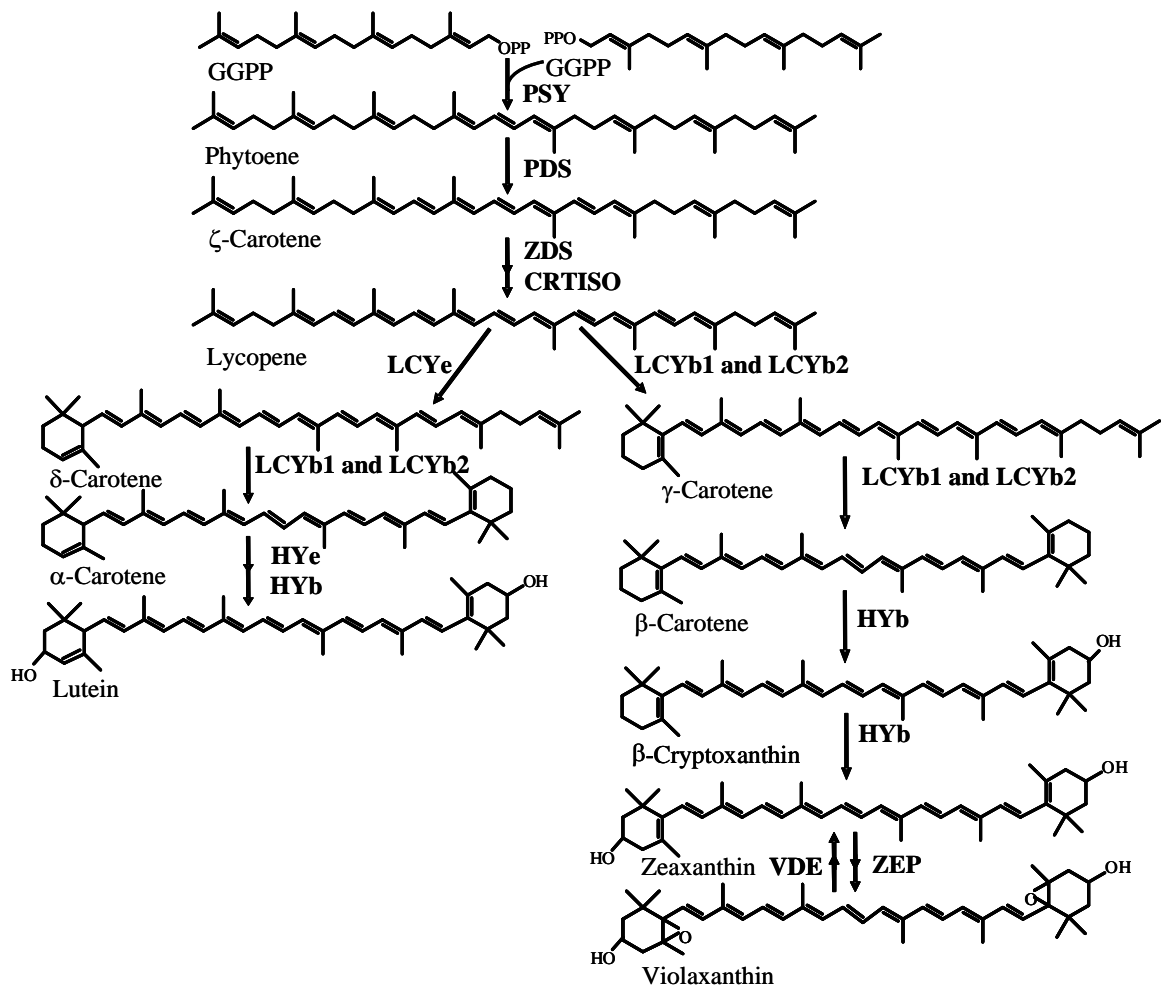


Fig. 2

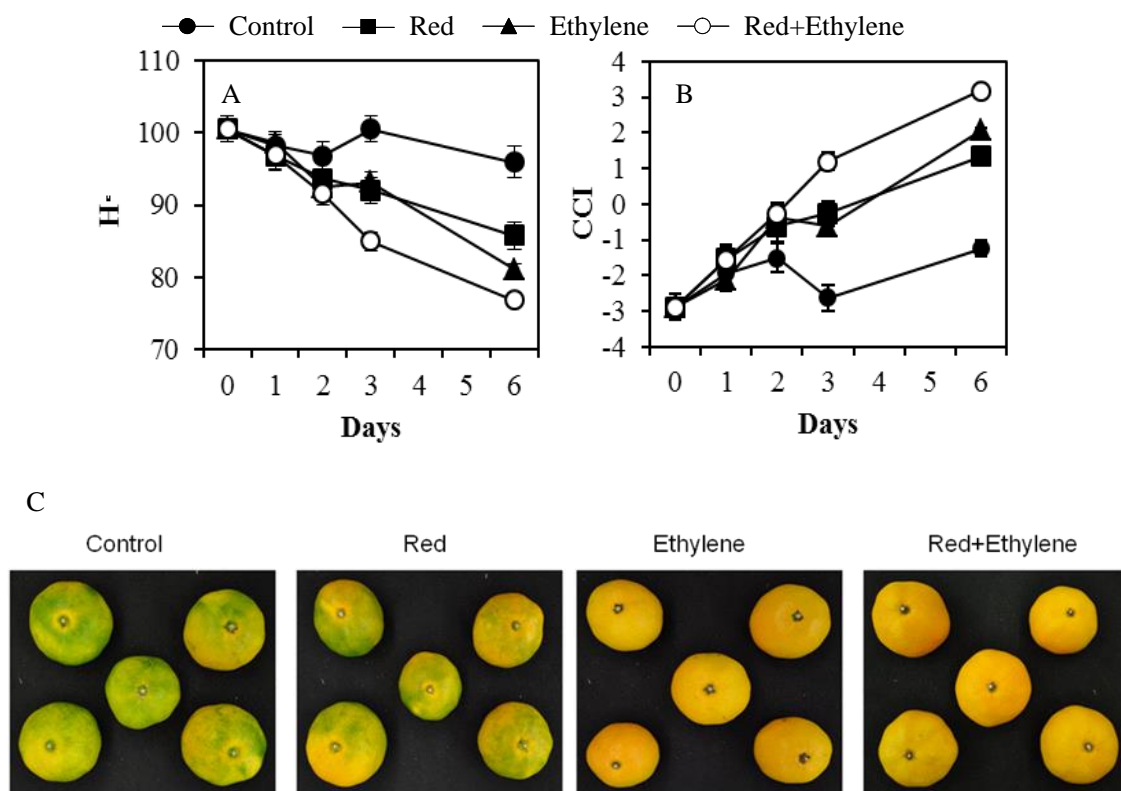


Fig. 3

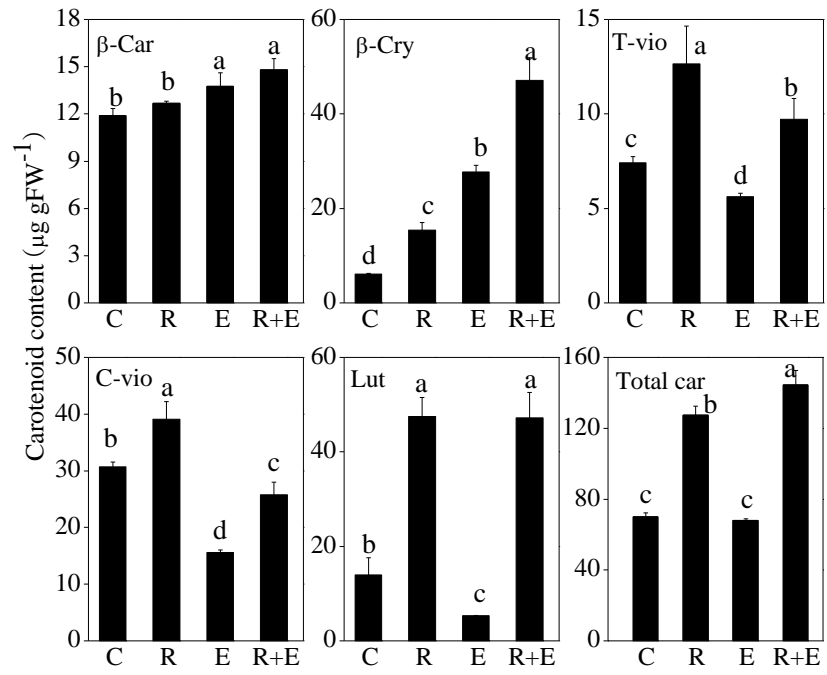


Fig. 4

