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Short communication

UV-B treatment controls chlorophyll degradation and related gene expression in broccoli (*Brassica oleracea* L. Italica Group) florets during storage

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ABSTRACT

Control of chlorophyll (Chl) degradation and related gene expression in response to UV-B treatment were investigated in broccoli florets during storage. Reductions in hue angle value and Chl content in broccoli florets during storage at 4, 15 and 25 °C were delayed with 19 kJ m^{-2} of UV-B irradiation. Furthermore, up-regulation of *BoSGR* and *BoNYC1*, Chl-degrading genes, was found with floret yellowing in the control during storage at 15 °C. In contrast, expression of those genes in UV-B-treated broccoli florets were effectively suppressed during storage, suggesting that UV-B treatment could be useful for quality maintenance of broccoli florets during storage.

1. Introduction

Deterioration of broccoli florets after harvest is mainly observed as floret yellowing, resulting from chlorophyll (Chl) degradation. In the putative Chl degradation pathway, several Chl-degrading enzymes, such as chlorophyllase, pheophytinase (PPH), pheophorbide *a* oxygenase (PAO) and Mg-dechelating substance, have been reported (Schelbert et al., 2009; Matile et al., 1999; Shioi et al., 1996). Chl-degrading peroxidase and its gene (*Chl-PRX*) expression are also involved in Chl oxidation of green plant tissues (Yamauchi, 2013; Aiamla-or et al., 2012; Ma et al., 2018). The senescence-associated genes including *STAY-GREEN* (*SGR*) were identified to encode chloroplast-located proteins and involved in Chl degradation (Park et al., 2007; Hörtensteiner, 2009; Sakuraba et al., 2015). Recently, Shimoda et al. (2016) reported that *SGR* in *Arabidopsis* encoded Mg-dechelatase, which removes the Mg atom from Chl *a*. In addition, the *NON-YELLOWING COLORING1* (*NYC1*) gene encoding Chl *b* reductase was isolated from *Arabidopsis* and reported to be involved in the Chl cycle by altering Chl *b* to 7-hydroxymethyl Chl *a* (Sato et al., 2015, 2018).

Low temperature storage is an efficient postharvest technology to suppress plant metabolism processes, such as respiration rate, ethylene production, and protein synthesis or degradation (Gao et al., 2018). Postharvest treatments, including those with chemicals, low or high temperature and UV irradiation, also have been reported to delay broccoli senescence (Gómez-Lobato et al., 2012, 2014; Perini et al., 2017). In our previous papers, broccoli florets with an optimal UV-B treatment of 19 kJ m^{-2} suppressed yellowing during storage at 15 °C and effectively reduced Chl-degrading enzyme activities and resulting gene expressions (Aiamla-or et al., 2010, 2012).

In this paper, we further examined the effects of different storage temperatures on the efficiency of UV-B treatment to maintain the quality of postharvest broccoli. Furthermore, we addressed an inhibitory effect of UV-B treatment on relative expression levels of *SGR* and *NYC1* genes in stored broccoli florets.

2. Materials and methods

2.1. UV-B irradiation

Broccoli (*Brassica oleracea* L. Italica Group, cv Pixel) heads were harvested at Fukuoka Prefecture, Japan, transported to a laboratory at Yamaguchi University, and then immediately exposed to UV-B irradiation at a dosage of 19 kJ m^{-2}. The application of UV-B irradiation was previously described by Aiamla-or et al. (2010). Broccoli heads with or without UV-B irradiation were stored at 4, 15 and 25 °C, in darkness. For surface color analysis, six heads were used for each irradiation x storage temperature x storage time treatment. After color analysis, three heads were removed at scheduled intervals during storage and their floret tissues were analyzed for Chl content and gene expression.

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Table 1
Changes in chlorophyll content of broccoli florets with or without 19 kJ m^{-2} UV-B irradiation during storage at 15 °C.

Treatment	Days	(mg/100 gFW)		
		Total Chl	Chl *a*	Chl *b*
Control	0	94.40 ± 0.28^{a}	75.62 ± 0.20^{a}	18.78 ± 0.13^{a}
	2	99.57 ± 5.89^{a}	79.73 ± 4.68^{a}	19.83 ± 1.22^{a}
	4	29.85 ± 1.36^{d}	24.71 ± 1.17^{d}	5.13 ± 0.21^{d}
	6	14.49 ± 0.59^{e}	10.87 ± 0.48^{e}	3.61 ± 0.11^{d}
UV-B treatment	0	99.53 ± 3.99^{a}	79.34 ± 2.36^{a}	20.18 ± 1.75^{a}
	2	93.66 ± 2.75^{a}	74.60 ± 2.09^{a}	19.06 ± 0.67^{a}
	4	64.09 ± 5.23^{b}	51.94 ± 4.08^{b}	12.14 ± 1.15^{b}
	6	49.76 ± 2.41^{c}	40.39 ± 1.92^{c}	9.37 ± 0.51^{c}

Data are the mean ± SE (n = 3). Different letters within columns indicate significant differences ($p < 0.05$) based on the least significant difference (LSD) test.

2.2. Floret yellowing and chlorophyll content

The surface color of broccoli heads was measured by color meter (Nippon-denshoku NF777, Japan). Chl content was analyzed according to Moran (1982). Chl was measured spectrophotometrically at the wavelengths of 664 nm and 645 nm.

2.3. RNA extraction and real-time PCR

The florets of broccoli stored at 15 °C were collected every two days over a 6-day storage period, and immediately frozen with liquid nitrogen. The frozen florets were kept at −80 °C. Total RNA was extracted from 80 mg of frozen floret tissue, of which 1.5 µg was used for cDNA synthesis (Aiamla-or et al., 2012). Primers specific to the senescing associated gene, *BoSGR,* (accession no.DK463677, forward: 5′-TTCCGACAACCGAAGTAA-3′, reverse: 5′-GTGAGCGTATAAGT TGG-3′) were designed following Gomez-Lobato et al. (2014). For Chl *b* reductase (*BoNYC1*, accession no.EH428873*)*, primers were as according to Hasperué et al. (2013); forward: 5′-TTACATCTCGCAGTTC TGA-3′, reverse: 5′-GCAATACCAACTACCTTAGC-3′. The actin gene *BoActin* (accession no.AF044573, forward: 5′-CTTGCACCAAGCAT GAA-3′, reverse: 5′-AGAATGGAACCGATCCA-3′) was used as a normalizer. For gene expression analysis, cDNA was used as a template for two-step RT-qPCR reaction, using a StepOne Plus™ Real-Time PCR system (Applied Biosystems, Japan). To perform RT-qPCR, SYBR green PCR master mix (Applied Biosystems, Japan) was used as a dye. The thermal cycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C, 15 s and 60 °C, 1 min. Each gene expression level was shown as a ratio with the control expression on day 0.

2.4. Statistical analyses

Significance of differences for each parameter, such as hue angle value and gene expression, between the control and UV-B-treated broccoli was compared by *t*-test. In Table 1, the control and UV-B-treated broccoli together with storage period were subjected to analysis of variance (ANOVA), and the mean values were compared by the least significant difference (LSD) test at a significance level of $p < 0.05$ using SAS system version 9.0 software.

3. Results

3.1. Effects of UV-B treatment on appearance and hue angle value changes

Control broccoli florets stored at 25 °C turned green-yellow on day 3 and changed to yellow on day 5, whereas floret yellowing occurred

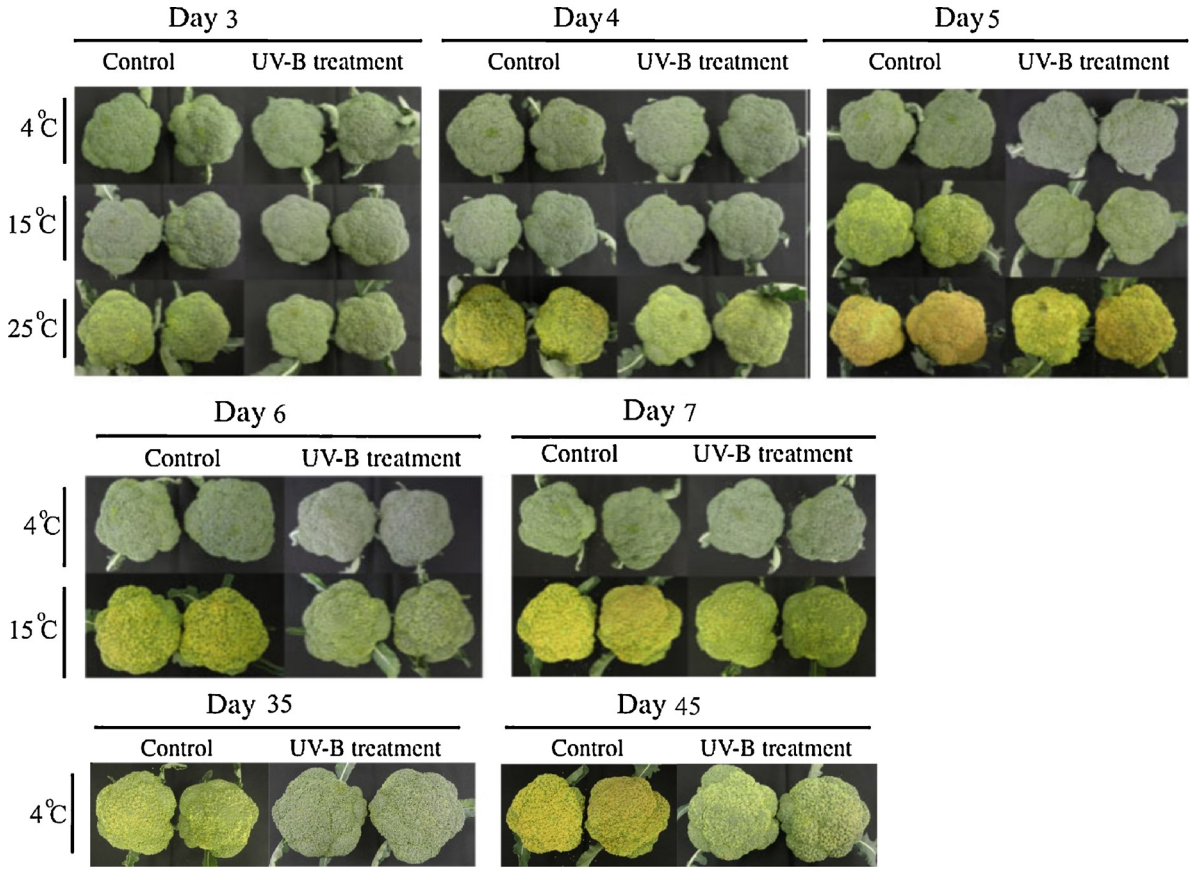

Fig. 1. Changes in the appearance of broccoli florets treated with or without 19 kJ m^{-2} UV-B irradiation during storage at 4, 15 or 25 °C.