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Expression of *Stay-Green* encoding gene (*BoSGR*) during postharvest senescence of broccoli

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ABSTRACT

Degreening caused by chlorophyll degradation is the most important feature that determines postharvest loss of quality in broccoli. Chlorophyll molecules are assembled to several thylakoid proteins, from which chlorophylls must be released in order to be catabolized. Stay-Green (SGR), a chloroplast-located protein, specifically interacts with light harvesting complex subunits helping toward their destabilization and to the release of chlorophylls. In this work, a fragment of a gene encoding a SGR from broccoli (*BoSGR*) was cloned. The expression of *BoSGR* was analyzed and detected an important increment during postharvest senescence, simultaneously with chlorophyll degradation. In order to analyze the effect of different growth regulators, different groups of broccoli heads were treated with cytokinins, ethylene and 1-MCP. Cytokinins and 1-MCP delayed the increment of *BoSGR* expression whereas ethylene accelerated the process. In addition, several postharvest treatments that delay degreening in broccoli florets were applied to evaluate their effects on *BoSGR* expression. Samples treated with modified atmosphere, hot air, UV-C or white lights showed a delay in chlorophyll degradation and degreening. In most cases, the treatments also delayed the increment of *BoSGR* expression during senescence, reaffirming the relationship between the expression of this gene and chlorophyll degradation.

1. Introduction

Broccoli (*Brassica oleracea* L. Italica Group) is a product that has recently a grown in demand with increased consumption due to its little caloric value and its high content of nutrients such as vitamins A and C, antioxidants, and fibers. In addition, recent studies have indicated that broccoli contains high levels of anti-carcinogenic compounds such as glucosinolates (Mukherjee et al., 2007).

Floral heads of broccoli are composed of hundreds of florets arranged in whorls on top of a stem. For consuming, they are harvested while they are still in development. Since inflorescences require high levels of nutrients, water and hormones, harvesting causes a severe stress, determining accelerated senescence (Chen et al., 2008; Downs et al., 1997). Yellowing is the main sign of senescence and, in the case of broccoli and other green horticultural products, determines commercial quality.

Broccoli senescence is delayed by cytokinins and promoted by ethylene. Treatments with cytokinins delay the physiological changes that usually accompany the senescence of florets (Downs et al., 1997). On the contrary, ethylene seems to be the principal promoter of senescence and yellowing (King and Morris, 1994). In this sense, treatments with 1-MCP, an inhibitor that binds irreversibly to ethylene receptor, can delay yellowing and extends the shelf-life (Gong and Mattheis, 2003; Ku and Wills, 1999; Yuan et al., 2010).

Several postharvest treatments like hot air (Costa et al., 2005a, 2006), UV-C (Büchert et al., 2011b; Costa et al., 2005b), modified atmosphere (Eason et al., 2007) or visible light (Büchert et al., 2011c) have showed their effectiveness in delaying degreening.

Yellowing is caused by chloroplast disassembling and chlorophyll catabolism (Fukasawa et al., 2010). The chlorophyll degradation pathway can be divided into early steps, which occur in the chloroplast, followed by species-specific modification of

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chlorophyll breakdown products which are stored in the vacuole. Chlorophyll molecules are located in the thylakoid membranes inside the chloroplasts. During senescence, thylakoid membranes are disassembled and chlorophylls are released and catabolized (Matile et al., 1999; Schelbert et al., 2009).

A few years ago, screening for stay-green mutants in many species allowed uncovering a novel chloroplast-located protein, termed Stay-Green (SGR) (Hörtensteiner, 2009), whose function is related to chlorophyll breakdown, although it is not considered a chlorophyll catabolic enzyme itself. SGR was shown to specifically interact with light harvesting complex subunits of photosystem II (LHCII) (Park et al., 2007). It is assumed that SGR interaction with LCHII may trigger destabilization and the subsequent degradation of both chlorophyll and apoproteins (Hörtensteiner, 2009; Park et al., 2007). It has been observed that the absence of SGR during senescence indirectly causes retention of chlorophyll in stable apoproteins (Park et al., 2007).

In this study, it is hypothesized that the increase of expression of genes encoding SGR will enhance chlorophyll degradation and yellowing of broccoli florets. Thus, the objective of the present work was to characterize the expression of a gene encoding a putative SGR (*BoSGR*) during postharvest senescence of broccoli and to analyze the effect of several treatments that modifies senescence rate on *BoSGR* expression.

2. Materials and methods

2.1. Plant material

Broccoli (*B. oleracea* var. *Italica*; cv. *Cicco*) heads were obtained from a local producer in La Plata, Argentina and immediately transported to the laboratory. Leaves were also obtained from plants of the same farm.

2.2. Senescence treatments

Heads were separated in different parts (see Fig. 1a) and stored together with leaves in a well-ventilated chamber isolated from external light at 22 °C for 120 h. Samples were taken at 0 h and 120 h. After that, samples were cut, frozen in liquid nitrogen and stored at −20 °C until analysis.

Another group of heads was placed in plastic cups containing a small amount of distilled water to prevent dehydration and stored as described above for 120 h. Heads were sampled periodically for color analysis. After that, the florets of five heads were separated from stems, randomly grouped and frozen in liquid nitrogen and stored at −20 °C until analysis.

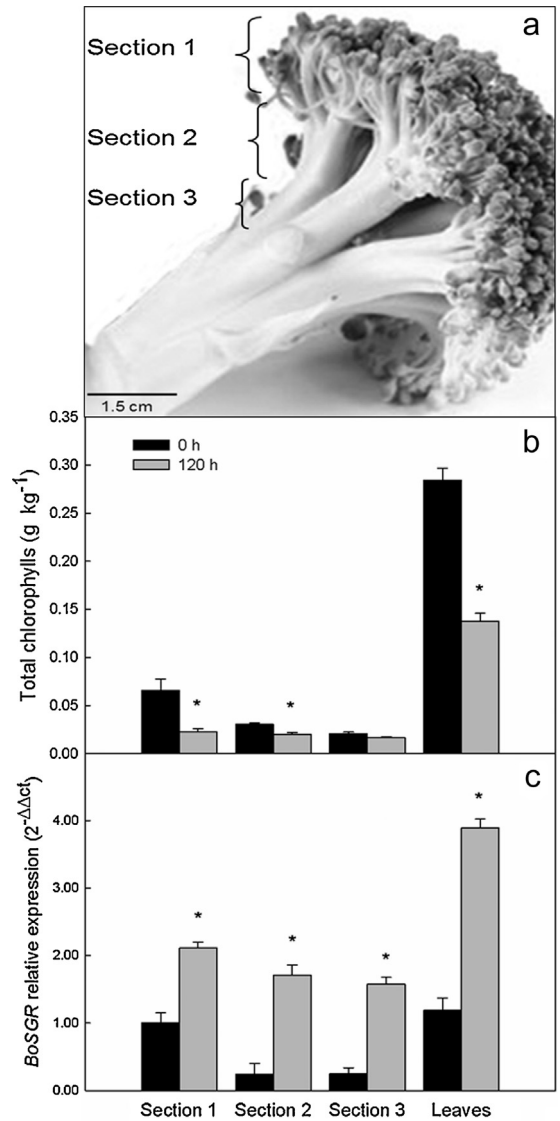

Fig. 1. Sectioning of broccoli florets (a). Total chlorophyll content in different sections of broccoli florets at day 0 and day 5 of induced senescence (b). Gene expression assessment of *BoSGR* during senescence of different sections of broccoli florets and leaves. Values of section 1 at 0 h were used as reference sample (calibrator) (c). Asterisks show statistical differences between 0 and 120 h in the same section ($P < 0.005$).

2.3. Hormone treatments

Whole broccoli heads were immersed for 10 min in solutions containing 100 mg kg^{-1} 6-bezylaminopurine (6-BAP) with 0.1% DMSO; 100 mg kg^{-1} 2-choloethylphosphonic acid (Ethephon, an ethylene-releasing agent) with 0.1% DMSO; or 0.1% DMSO as control (Costa et al., 2004).

For 1-MCP treatment, the heads were placed in plastic bowls containing a small amount of distilled water and treated with 1-MCP (1 μL L^{-1}) in a hermetically sealed container for 16 h at 22 °C. Controls were kept under the same conditions without 1-MCP. Thirty heads were used for each condition. After treatment, samples were placed in plastic cups containing a small amount of distilled water to prevent dehydration and stored as described above. Samples were collected at different times for color measurements. After that, six heads were chosen for each condition, separated from stems, frozen using liquid nitrogen and stored at −20 °C until use.

2.4. Physical treatments

Forty-five broccoli heads were used for each physical treatment. For heat treatments, heads were treated with hot air at 45 °C for 3 h (Costa et al., 2005a). The same number of heads without heating was used as controls. After treatment, the broccoli heads were loosely covered with PVC film to diminish water loss and stored at 22 °C. Samples were taken after 0 (initially for control samples and immediately following treatment for heat-treatment samples), 72 and 120 h of storage. Then, individual florets were removed and frozen in liquid nitrogen and stored at −20 °C until analysis.

For the modified atmosphere treatment, heads were individually and hermetically covered with a polyethylene bag (40 μm thick, 20 cm × 30 cm) and stored in darkness at 22 °C for 120 h (Gomez-Lobato et al., 2012a). The same number of heads were loosely covered with PVC film and utilized as controls. Samples were taken and processed as mentioned above.

In the case of UV-C treatment, broccoli heads were placed vertically in plastic trays, in order to assure homogeneous irradiation of florets, and put under a bank of 4 UV-C lamps (TUV G30T8, 30 W, Philips) (Costa et al., 2005b). The heads were irradiated at a distance of 30 cm to obtain a dose of 10 kJ m^{-2}. The flux intensity of lamps was measured with a digital radiometer (Cole-Palmer Instrument Company, Vernon Hills, IL, USA). The same number of heads without irradiation was utilized as controls. After treatment, the broccoli heads were also loosely covered with PVC and stored at 22 °C for 120 h in darkness. Samples were taken and processed as mentioned above.

For the visible light treatments, stems of heads were individually placed in plastic cups containing a small amount of distilled water to prevent dehydration. Samples were put in a well-ventilated chamber isolated from external light sources. One half of the chamber was kept in complete darkness (<1 μmol m^{-2} s^{-1}) and the other half was exposed to 12 μmol m^{-2} s^{-1} continuous light intensities. Samples were collected and processed as mentioned previously.

2.5. Superficial color measurement

Superficial color was evaluated by measuring the parameters *a* and *b* in five positions for each broccoli head with a chromameter (Model CR-300, Minolta, Osaka, Japan). Hue angle ($h°$) was calculated as $h° = \tan^{-1}(b/a)$ when $a > 0$ and $b > 0$ or as $h° = 180° - \tan^{-1}(b/a)$ when $a < 0$ and $b > 0$.

2.6. Determination of chlorophyll content

Frozen broccoli florets were ground in liquid nitrogen and 0.5 g of the resulting powder was mixed with 5 mL of 80% (v/v) acetone and centrifuged at 10,000 × *g* for 10 min at 4 °C. Chlorophyll content was measured in supernatant by spectrophotometry (Inskeep and Bloom, 1985) and results were expressed as g of total chlorophyll per kilogram of fresh tissue. All measurements were performed in quintuplicate.

2.7. RNA extraction and real-time PCR

Florets from broccoli at various senescent stages and different treatments were ground in liquid nitrogen, and total RNA was obtained by hot borate method (Wan and Wilkins, 1994) and quantified by UV spectrophotometry. An amount of about 6 μg of total RNA were treated with RQ1 DNAse (Promega), purified with chloroform:1-octanol (24:1) and precipitated with 3 M sodium acetate. Purified RNA was quantified again and 4 μg were used for cDNA synthesis using MML-V reverse transcriptase (Promega) and random primers (hexamers). Resulting cDNA was employed as a template for two-step qPCR reaction using an StepOnePlus™ Real-Time PCR System (Life Technologies) and FastStart Universal SYBR Green Master (Roche). Actin (AF044573) was used as normalizer, forward: 5′-CCAGAGGTCTTGTTCCAGCCATC-3′ and reverse: 5′-GTTCCACCACTGAGCACAATGTTAC-3′. Primers specific to *BoSGR* were designed from sequence *NYE* from *B. oleracea* (GeneBank accession number: DK463677): forward: 5′-TTCCGACAACCGAAGTAA-3′ and reverse: 5′-GTGAGCGTATAAGTTCTTGG-3′. The following program was used: one cycle at 95 °C for 10 min, then 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Real-time fluorescence data were captured at the end of each cycle of 15 s. Melt-curve analysis was conducted by holding the samples at 95 °C for 15 s, then reducing the temperature to 60 °C for 1 min, and then increasing the temperature to 95 °C for 15 s. During this change of temperature fluorescence was measured continuously. Negative controls consisting of primers with no template were included. Each measurement was performed in triplicate.

2.8. Statistical analysis

Experiments were performed according to a factorial design. Data were analyzed using ANOVA, and the means were compared by the LSD test at a significance level of 0.05.

3. Results

3.1. Cloning of BoSGR in broccoli

Broccoli undergoes intense degreening during postharvest senescence due to chlorophyll degradation. In Arabidopsis, SGR is considered to be related to chlorophyll catabolism by specifically interacting with light-harvesting complex subunits of photosystem II (LHCII) (Hörtensteiner, 2009). This gene was termed firstly SID (*senescence-induced degradation*) in *Festuca pratensis* (Thomas, 1987), but ortholog genes from rice, pea and *Arabidopsis* have now been designated SGR (STAY-GREEN) (Jiang et al., 2007; Park et al., 2007; Sato et al., 2007), SGN (Park et al., 2007) or NYE1 (*nonyellowing*) (Ren et al., 2007). An ortholog of SGR in broccoli was sought for analysis in this research. This was accomplished by performing a search that comparing the published sequence of NYE1 for Arabidopsis (Ren et al., 2007) against public EST database of *B. oleracea* using current web-base tools (Altschul et al., 1997). An 872 bp sequence (DK463677) from *B. oleracea* EST database was identified that matched a sequence *NYE1* from *Arabidopsis thaliana* (*AtNYE1*; NM118421.3). Using specific primers for this sequence, a 199 bp cDNA was isolated from broccoli and sequenced, and, after its identity with *AtNYE1* (85%) and *NYE1* of *Brassica rapa* subsp. *campestris* (97%) was verified, it was named *BoSGR*.

3.2. BoSGR expression in different section of broccoli florets

Different sections of broccoli heads and leaves were incubated at 22 °C in darkness in order to evaluate the expression of *BoSGR*. Initially and after five days, heads were segmented into section 1 (inflorescences), section 2 (small branchlets) and section 3 (main floret stem) (Fig. 1a). The progress of senescence was evaluated by measuring the chlorophyll content. Higher chlorophyll content was detected in section 1 (inflorescences) in relation with the other two sections (2 and 3), which had similar chlorophyll levels. Furthermore, the content of this pigment showed a decrease during senescence. In leaves, chlorophyll content was much higher in comparison to that of florets and also showed a drop after 5 d of senescence (Fig. 1b).

Expression of *BoSGR* was analyzed by qRT-PCR. We observed that after 120 h of incubation the transcripts accumulation of *BoSGR* was higher than at harvest (Fig. 1c).

3.3. BoSGR expression during broccoli postharvest senescence

As the main change in *BoSGR* expression during senescence was detected in section 1 (inflorescences), we decided to analyze the expression with more detail in this tissue. Broccoli heads were incubated as described previously but, in this new set of experiments, samples were taken at 0 h (initials) and after 72 and 120 h of incubation. During this period, senescence was followed by superficial color and chlorophyll content. The *Hue* angle values remained almost without changes until 72 h and then diminished significantly after 120 h of incubation (Fig. 2), which coincided with the yellowing of samples. Correspondingly, we observed that chlorophyll levels diminish continuously until the end of the experiment (120 h). Values of total chlorophylls were approximately 67% lower than that observed in pre-senescent tissues (Fig. 2). Differently, the

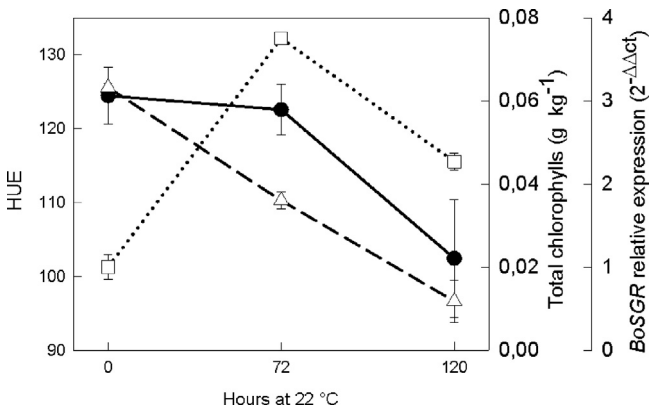

Fig. 2. *Hue* angle (●), chlorophyll content (△) and *BoSGR* expression (□) of broccoli heads stored during five days at 22 °C. *Hue* angle was calculated as described in Methods and chlorophyll contents are expressed as mass of chlorophyll per fresh weight mass of tissue, g kg^{-1}. Values of *BoSGR* expression of controls at 0 h were used as reference sample (calibrator).

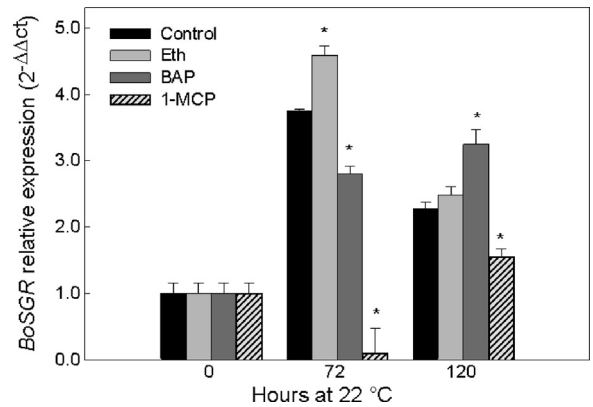

Fig. 3. Relative *BoSGR* expression of hormone-treated broccoli heads during five days of induced senescence at 22 °C. Values of *BoSGR* expression of controls at 0 h were used as reference sample (calibrator). Asterisks show statistical differences between each treated samples and controls at the same time ($P < 0.005$).

expression of *BoSGR* increased continuously up to 72 h and then decreased at 120 h (Fig. 2).

3.4. Effects of hormone treatment on BoSGR expression

Senescence is mainly regulated by the hormones cytokinins and ethylene, which respectively inhibit and promote this process. Several previous studies have shown that 1-MCP, an inhibitor of ethylene action, can delay degreening and chlorophyll degradation. The effect of these hormones on *BoSGR* expression was analyzed in this study. In controls, the *Hue* angle decreased slightly after 72 h of storage, but the decrease was more pronounced after 120 h (Table 1). Samples treated with BAP maintained the *Hue* angle and their green color until the end of treatment. Samples treated with 1-MCP also showed a decrease in *Hue* angle but these values were higher than those of controls. On the contrary, ethephon treatment caused a higher decrement in *Hue* values. The chlorophyll levels were also measured during the incubation at 22 °C (Table 1). Total chlorophylls of control, BAP and ethephon treatment started decreasing after 72 h, and diminished continuously during incubation. Differently, total chlorophylls levels were maintained for 72 h in samples treated with 1-MCP. Reduction of chlorophylls started only after 72 h, which led to higher chlorophyll levels than controls at the end of storage period.

As was described previously, the expression of *BoSGR* in controls increased until 72 h and then decreased. In ethephon treated samples, the expression of *BoSGR* was higher than that of controls at 72 h but showed values similar to controls at the end of treatment. In contrast, the treatment with BAP causes a lower expression at 72 and 120 h with respect to controls (Fig. 3).

In samples treated with 1-MCP, mRNA levels were significantly lower than controls at 72 h and 120 h (Fig. 3).

3.5. Effect of postharvest physical treatments on BoSGR expression

Broccoli floret heads were treated with different postharvest physical treatments to evaluate their effect on *BoSGR* expression. Previous studies have demonstrated that treatments with hot air (Costa et al., 2005a, 2006) and modified atmosphere (Eason et al., 2007) can delay senescence and chlorophyll degradation in broccoli. In this study, we performed these treatments according to description given in material and methods and stored the samples at 22 °C. In controls, the *Hue* values remained unchanged for 72 h and then decreased, while samples treated with hot air or modified atmosphere maintained higher *Hue* values until the end of experiment (Table 2). The levels of chlorophyll decreased in control samples. Significantly, florets maintained in modified atmosphere almost completely retained their chlorophyll during

Table 1
Hue values and total chlorophyll content (total Chl per fresh weight mass of tissue, g kg^{-1}) in controls and hormone-treated broccoli florets during postharvest senescence at 22 °C.

	Hue				Total chlorophyll			
	Control	Ethephon	BAP	1-MCP	Control	Ethephon	BAP	1-MCP
0 h	124.25 ± 2.89	124.25 ± 2.89	124.25 ± 2.89	124.25 ± 2.89	0.160 ± 0.011	0.160 ± 0.011	0.160 ± 0.011	0.160 ± 0.011
72 h	124.91 ± 3.86	121.00 ± 3.84*	127.37 ± 2.65*	124.64 ± 5.69*	0.093 ± 0.01	0.080 ± 0.012*	0.101 ± 0.007*	0.157 ± 0.004*
120 h	102.63 ± 8.09	98.79 ± 2.44*	127.06 ± 2.51*	117.25 ± 4.86*	0.056 ± 0.01	0.034 ± 0.002*	0.094 ± 0.036*	0.109 ± 0.002*

* Statistical differences between each treated samples and controls at the same time ($P < 0.005$).

Table 2
Hue values and total chlorophyll content (total Chl per fresh weight mass of tissue, g kg^{-1}) in controls, modified atmosphere and heat treated broccoli florets during postharvest senescence at 22 °C.

	Hue			Total chlorophyll content		
	Control	AM	TT	Control	AM	TT
0 h	124.43 ± 3.89	124.43 ± 3.89	124.43 ± 3.89	0.0632 ± 0.001	0.0632 ± 0.001	0.0632 ± 0.001
72 h	122.55 ± 3.41	123.49 ± 2.92	132.02 ± 2.12*	0.0360 ± 0.002	0.0618 ± 0.004*	0.0434 ± 0.007*
120 h	102.42 ± 7.94	115.11 ± 7.92*	105.15 ± 2.72*	0.0118 ± 0.005	0.0186 ± 0.001*	0.0735 ± 0.003*

* Statistical differences between each treated samples and controls at the same time ($P < 0.005$).

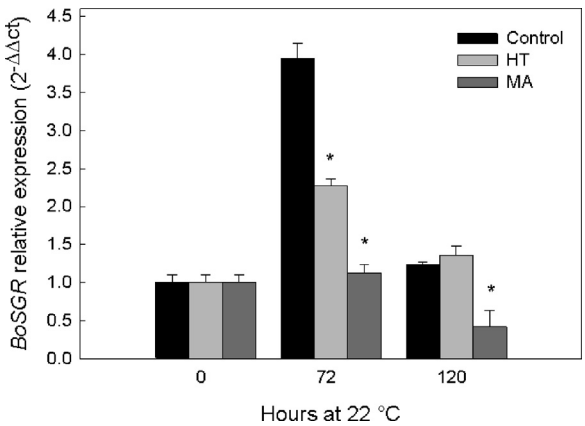

Fig. 4. Relative *BoSGR* expression of broccoli florets subjected to heat or modified atmosphere treatments during 5 d of induced senescence at 22 °C. Values of *BoSGR* expression of controls at 0 h were used as reference sample (calibrator). Asterisks show statistical differences between each treated samples and controls at the same time ($P < 0.005$).

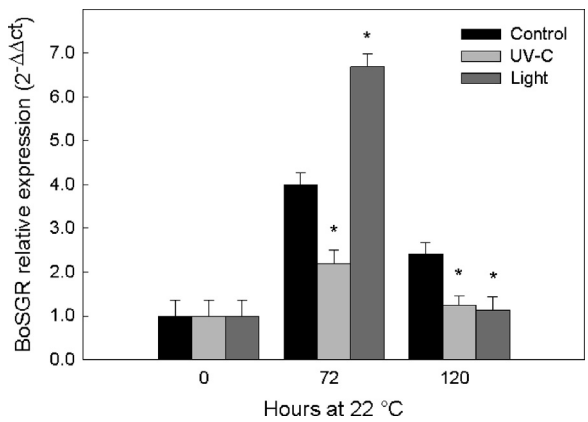

Fig. 5. Relative *BoSGR* expression of broccoli florets subjected to UV-C or white light treatments during five days of induced senescence at 22 °C. Values of *BoSGR* expression of controls at 0 h were used as reference sample (calibrator). Asterisks show statistical differences between each treated samples and controls at the same time ($P < 0.005$).

storage incubation. Differently, although samples treated with hot air degraded chlorophyll, they had higher contents than controls after 72 and 120 h (Table 2).

The expression of *BoSGR* in controls increased after 72 h and then decreased at the end of the treatment. Samples treated with hot air showed lower values of relative expression at 72 and 120 h in relation to controls. In the case of samples treated with modified atmosphere, they showed a significant decreased in *BoSGR* expression in comparison to controls during the incubation (Fig. 4).

Treatments with different kinds of radiation were performed to evaluate their effects on *BoSGR* expression. Previous studies have demonstrated that treatments with UV-C (Costa et al., 2005b) or light (Büchert et al., 2011c) can delay broccoli senescence. Results in this study show that in controls, superficial color decreased continuously during storage for 120 h. In contrast, UV-C and light treatments *Hue* values slightly decreased at 72 h of storage and then continuous decreased until 120 h but the *Hue* values are higher compared with controls and yellowing was less evident (Table 3). In all samples, the levels of chlorophyll decreased during storage at 22 °C. In light and UV-C treated samples, the content of chlorophyll was higher than in controls after 72 and 120 h, showing light treatment more effective in retention of chlorophyll (Table 3).

As in other cases, expression of *BoSGR* increased after 72 h and then decreased at the end of the storage. Heads treated with UV-C showed a greater decrease at 72 and 120 h compared with controls. Differently, samples treated with visible light showed an increase after 72 h and then a significant decrease in *BoSGR* after 120 h in relation to controls (Fig. 5).

4. Discussion

Degreening detected during senescence is caused by chlorophyll degradation, which is carried out by several catabolic enzymes. However, before the enzymes can act, it is necessary that chlorophyll be released from the LHC. Recent studies indicate that SGR genes encode a chloroplast protein that is required for the initiation of chlorophyll breakdown (Aubry et al., 2008; Hörtensteiner, 2009; Park et al., 2007).

The yellowing that occurs during postharvest senescence of broccoli is an important feature for this horticultural crop and is an essential determinant of commercial quality. As a consequence, characterization of genes related to chlorophyll catabolism is highly relevant in broccoli. In this work, a search of public databases found a sequence of 872 bp that belongs to *B. oleracea* (DK463677). The fragment showed similarities to other genes encoding for SGR and was named *BoSGR*.

Several studies have shown that SGR expression is induced at the onset of leaf senescence or fruit ripening, concomitant with chlorophyll breakdown (Borovsky and Paran, 2008; Yang et al., 2009). Similarly, it was found that the expression of *BoSGR* increased during postharvest senescence of broccoli in all parts analyzed. In florets, chlorophylls were continuously degraded but the expression of *BoSGR* increased for 3 d and then decreased, probably due to a regulatory mechanism that detects the substrate absence. The increment of *BoSGR* expression was detected not only in senescent florets but also during leaf senescence.

It was previously shown that chlorophyll degradation is regulated positively by ethylene and negatively by cytokinins during broccoli senescence (Costa et al., 2004; Downs et al., 1997; Gomez-Lobato et al., 2012a; Gong and Mattheis, 2003). In this sense, 1-methylcyclopropene (1-MCP; an inhibitor of ethylene action) delayed broccoli yellowing and decreased chlorophyll-degrading activities (Gomez-Lobato et al., 2012b; Gong and Mattheis, 2003; Kasim et al., 2007; Ku and Wills, 1999; Yuan et al., 2010). This study found that ethylene enhanced *BoSGR* transcript levels, while BAP diminished the expression peak detected at 72 h. Finally, treatment with 1-MCP greatly inhibited expression of *BoSGR*, in concordance with the effect of ethylene. The effect on BoSGR expression clearly correlates with the effect of hormones on chlorophyll degradation. In this sense, the higher chlorophyll retention coincides with the lower BoSGR expression detected in 1-MCP treated heads. This pattern of expression is similar to those observed previously in other chlorophyll degrading genes like *BoPPH*, *BoPaO* and *BoRCCR* (Büchert et al., 2011a; Gomez-Lobato et al., 2012a, 2012b).

Several studies have focused on the use of postharvest treatments to maintain the green color and reduce chlorophyll loss during postharvest senescence (Costa et al., 2006; Ku and Wills, 1999; Yamauchi et al., 1997; Zaicovski et al., 2008). The use of films that generate modified atmospheres (Barth et al., 1993; Büchert et al., 2011b; Eason et al., 2007; Gomez-Lobato et al., 2012a) or different heat treatments (Büchert et al., 2011b; Costa et al., 2005a, 2006; Funamoto et al., 2002; Gomez-Lobato et al., 2012a; Lemoine et al., 2009) have been successfully employed to delay senescence. This study found that hermetic enclosure of heads in polyethylene bags reduced chlorophyll degradation and completely inhibited the peak of *BoSGR* expression and that heat treatment reduced the peak of expression after 72 h of incubation. It was shown that these treatments can also inhibit or delay the increase of expression of other genes associated to chlorophyll catabolism. Previously it has

Table 3
Hue values and total chlorophyll content (total Chl per fresh weigh mass of tissue, g kg^{-1}) in controls, UV-C and light treated broccoli florets during postharvest senescence at 22 °C.

	Hue			Total chlorophyll content		
	Control	UV-C	Light	Control	UV-C	Light
0 h	118.60 ± 7.07	118.60 ± 7.07	118.60 ± 7.07	0.0746 ± 0.01	0.0746 ± 0.01	0.0746 ± 0.01
72 h	88.41 ± 6.59	109.75 ± 1.35*	112.60 ± 10.93*	0.0204 ± 0.002	0.0512 ± 0.007*	0.0541 ± 0.005*
120 h	83.72 ± 6.39	102.86 ± 2.15*	96.36 ± 9.69*	0.0164 ± 0.001	0.0219 ± 0.001*	0.0385 ± 0.006*

* Statistical differences between each treated samples and controls at the same time ($P < 0.005$).

been found that modified atmosphere also inhibits the expression of *BoPaO* (Gomez-Lobato et al., 2012a). Heat treatments can delay the increment of chlorophyll catabolic enzyme activities, including chlorophyllase, Mg-dechelatase, Chl-degrading peroxidase and chlorophyll oxidase (Costa et al., 2006; Funamoto et al., 2002) as well as the expression of *BoPaO* (Gomez-Lobato et al., 2012a). In the case of UV-C, this treatment also provokes a delay in chlorophyll degradation, which was associated with a reduction of chlorophyll degrading enzymes activities (Costa et al., 2005b).

In this study, UV-C treated samples showed a lower expression of *BoSGR* during incubation as it was detected previously with *BoCHL2* (Büchert et al., 2011b), *BoPPH* (Büchert et al., 2011a) and *BoPaO* (Gomez-Lobato et al., 2012a).

Darkness strongly induces senescence. In a previous work, it was demonstrated that storage of broccoli under continuous light delays postharvest senescence (Büchert et al., 2011c; Gomez-Lobato et al., 2012a). Moreover, it was also shown that the increase of *BoCHL2* expression is inhibited or delayed by light (Büchert et al., 2011b). Surprisingly, an important increment in *BoSGR* expression after 72 h of incubation was detected despite the significant delay in the degradation of chlorophylls. Probably, the inhibition of other genes involved in chlorophyll catabolism rather *BoSGR* contributed to maintenance of green color under visible light.

Given the phylogenetic similarity between *A. thaliana* and *B. oleracea*, an analysis was conducted by using the PLACE program (http://www.dna.affrc.go.jp/PLACE) to analyze AtNYE1 promoter (NM118421.3) (see supplementary figure and table). Among others, elements of response to ethylene were found, indicating a possible relation to the effect of ethylene on *BoSGR* expression as observed in this work. Furthermore, many of the post-harvest treatments cause a reduction in ethylene production (Amodio and Colelli, 2008; Burana and Srilaong, 2010; Pongprasert et al., 2011), which in turn could lead to lower expression of *BoSGR*. Numerous response elements to light were also found, which could explain the significant increase in the expression of *BoSGR* in heads stored under continuous light.

In conclusion, a fragment of a gene encoding a putative SGR protein (named *BoSGR*) from broccoli was cloned and the expression of the corresponding gene in different parts of the inflorescence during senescence was characterized. The expression was also analyzed in samples exposed to different hormonal and physical treatments that affect chlorophyll degradation. In general, treatments that delay chlorophyll degradation showed a lower *BoSGR* expression, which support a close relationship between chlorophyll catabolism and SGR.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.04.010.

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