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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.

### 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.

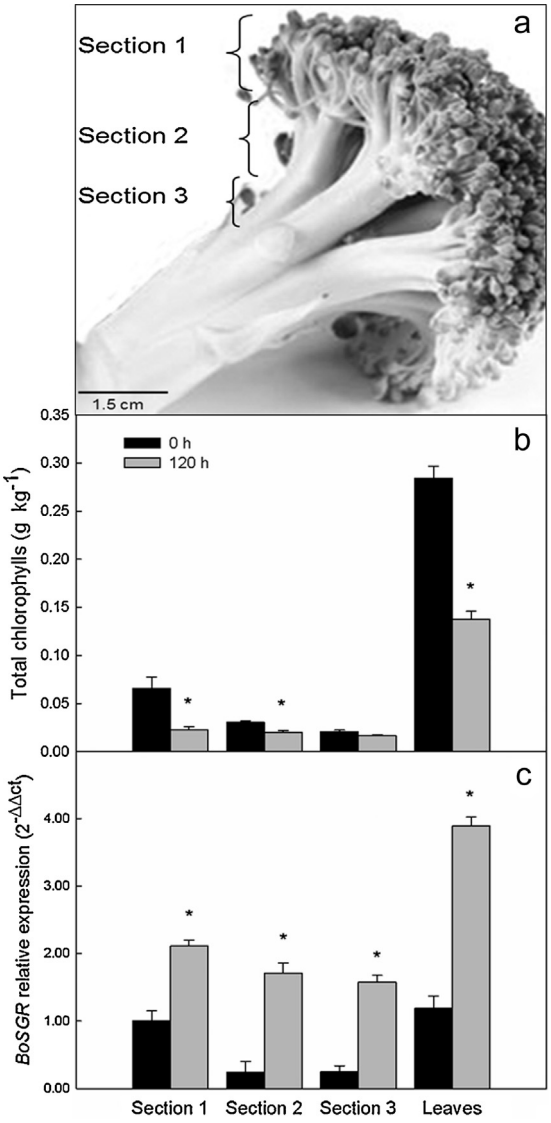


**Fig. 1.** Sectioning of broccoli florets (a). Total chlorophyll content in different sections of broccoli florets at day 0 and day 5 of induced senescence (b). Gene expression assessment of *BoSGR* during senescence of different sections of broccoli florets and leaves. Values of section 1 at 0 h were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

### 2.4. 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.