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Expression of a lipoxygenase encoding gene (*BoLOX1*) during postharvest senescence of broccoli

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ABSTRACT

Lipoxygenases (LOX) belong to a large family of plant enzymes that catalyze the hydroperoxidation of polyunsaturated fatty acids. Most of them are expressed during senescence and contribute to membrane deterioration and biosynthesis of jasmonic acid, a known senescence enhancer. In this work, we cloned a fragment of a gene encoding a LOX from broccoli (*BoLOX1*). The analysis of the sequence revealed that *BoLOX1* is closely related to other LOX from higher plants. Furthermore, we analyzed the expression of *BoLOX1* and detected a larger increase during postharvest senescence. A slight increase of total lipoxygenase activity was also found during senescence. In other sets of experiments, broccoli heads were treated with plant hormones, such as cytokinin and ethylene, as a way to assess the effect of such compounds on the expression of *BoLOX1*. Cytokinin treatment delayed the increase of *BoLOX1* expression and lipoxygenase activity whereas ethylene accelerated both processes. Also, several postharvest treatments were applied in order to delay senescence in broccoli florets and to evaluate their effects on *BoLOX1* expression. Samples treated with modified atmosphere, hot air, UV-C or white light showed a delay in chlorophyll degradation and degreening. In most cases, the treatments also delayed the increase of *BoLOX1* expression, reaffirming the relationship between the expression of this gene and senescence. However, treatments like modified atmospheres and visible light markedly increased lipoxygenase activity, which suggests a lack of correlation between *BoLOX1* expression and lipoxygenase activity.

1. Introduction

Lipoxygenases (LOX; EC 1.13.11.12) are enzymes involved in the degradation of free fatty acids and esterified lipids. LOXs catalyze the oxygenation of polyunsaturated fatty acids (PUFAs) to produce hydroperoxides, which in turn are transformed either chemically or enzymatically into a group of biologically active compounds known as oxylipins. These compounds have been implicated in several physiological roles such as signaling, pathogen and abiotic stress response, growth and development (Feussner and Wasternack, 2002; Porta and Rocha-Sosa, 2002; Wasternack, 2007). One of main products of this pathway is jasmonic acid, which, together with its methylated form, is an important plant hormone. Jasmonic acid has been proposed to be involved mainly in defense against pests and pathogens and as an intermediate in wounding responses (Blokhina et al., 2003), but it also has been postulated to be an accelerator of fruit ripening (Hu et al., 2009) and to have a role in leaf senescence (He et al., 2002).

Besides its role in the synthesis of oxylipins and jasmonic acid, LOXs have been associated with membrane deterioration in plant tissues through peroxidation of poly-unsaturated fatty acids (Cacas et al., 2005). Such oxidative activity results in membrane deterioration, loss of compartment and cell breakdown, being a key process in senescence (Zhuang et al., 1997). In addition, superoxide radicals and hydroperoxides produced by LOXs can also be involved in other aspects of cell degradation (Baysal and Demirdöven, 2007).

In Arabidopsis, six genes encoding LOX have been found, but physiological functions of only four of these genes have been described (*AtLOX1*, *AtLOX2*, *AtLOX3* and *AtLOX4*). It was shown that recombinant expressed proteins AtLOX1 and AtLOX5 have comparable oxygenase activity with either linoleic acid or linolenic acid, while AtLOX2, AtLOX3, AtLOX4 and AtLOX6 displayed a selective oxygenation of linolenic acid (Bannenberg et al., 2009).

AtLOX1 has an enhanced expression during senescence (He et al., 2002) while *AtLOX2* seems to have a role in stress induced senescence (Seltmann et al., 2010). The other *LOX* genes, *AtLOX3* and *AtLOX4*, like *AtLOX2*, contain chloroplast transit peptide sequences and are believed to be plastidial. *AtLOX3* and *AtLOX4* have been discussed to be involved in leaf senescence (He et al., 2002).

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Expression of *LOX*s in plants is regulated throughout development and also in response to wounding, water deficit and exposure to pathogens (Bell et al., 1995).

Heads of broccoli are composed of hundreds of florets arranged in whorls on top of stems. The inflorescences are harvested while the floral heads, branchlets and florets are totally immature, with the sepals completely surrounding the flower. Harvesting causes severe stress conditions due to nutrient and water deficiencies and changes in hormonal status, leading to an inability to maintain homeostasis and triggering the appearance of senescence symptoms (Page et al., 2001). One of the most visible symptoms of broccoli senescence is the degreening and yellowing of sepals accompanied by chlorophyll breakdown. Lipid degradation is another common feature of many tissues undergoing senescence. In senescing broccoli, the levels of polyunsaturated fatty acid (PUFA), PUFA hydroperoxides, and total fatty acid decline (Zhuang et al., 1994), while the activity of lipoxygenase and thiobarbituric acid-reactive substances (TBARS), which represent end-products from the peroxidation of lipids and other macromolecules, increase (Zhuang et al., 1995).

Taking into account the possible double role of LOXs during senescence: degradation of plasma membrane lipids and synthesis of jasmonic acid; the objective of the present work was to characterize the expression of a gene encoding a putative LOX (*BoLOX1*) during postharvest senescence of broccoli and to analyze the effect of several treatments that modifies senescence rate on *BoLOX1* expression.

2. Materials and methods

2.1. Plant material

Broccoli (*Brassica oleracea* var. *Italica*; cv. Cicco) heads and leaves were harvested at a local farm in La Plata, Argentina, and immediately refrigerated at 0 °C with ice, transported to the laboratory within two hours and processed.

2.2. Senescence treatments

Heads were separate in different parts (see Fig. 2a) and stored together with leaves in a well ventilated chamber isolated from external light at 22 °C with a relative humidity of 85% for 96 h. Samples were taken at 0 h and 96 h. After that, samples were cut, frozen in liquid nitrogen and stored at −20 °C until analysis (approximately three–four weeks).

Heads from another group were placed upright in plastic bowls with a small amount of distilled water with the stems touching the water to prevent dehydration and stored as described above for 120 h. Heads were sampled periodically for colour 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 (approximately three–four weeks).

2.3. Hormone treatments

Whole harvested broccoli heads were immersed for 10 min in solutions containing 0.1 g kg^{-1} 6-benzylaminopurine (6-BAP) with 1.1 g kg^{-1} DMSO; 0.1 g kg^{-1} 2-choroethylphosphonic acid (Ethephon, an ethylene-releasing agent) with 1.1 g kg^{-1} DMSO; or 1.1 g kg^{-1} DMSO as control (Costa et al., 2004). Thirty heads were used in each treatment condition. After treatment, samples were placed in plastic bowls containing a small amount of distilled water to prevent dehydration and stored as described above. Samples were collected at different times for colour measurements. After that, six heads were chosen from each condition and their florets were separated from stems, frozen in liquid nitrogen, and stored at −20 °C until analysis (approximately three–four weeks).

In the case of 1-MCP treatment, broccoli heads were placed in plastic bowls containing a small amount of distilled water and treated with this inhibitor (1 μL L^{-1}) in a hermetic container for 16 h at 22 °C. Controls were kept under the same conditions without 1-MCP. Samples were collected and processed as described above.

2.4. Physical treatments

Forty-five broccoli heads were used in each physical treatment. For hot air treatment, heads were placed in trays and covered with PVC film to diminish water loss. The heads were treated with hot air at 42 °C for 3 h (Costa et al., 2005a). The same number of heads without heating was utilized as controls. After treatment, the broccoli heads were loosely covered with PVC film to diminish water loss and stored as described above. Samples were taken after 0, 72 and 120 h of storage. Then, individual florets were removed, frozen in liquid nitrogen, and stored at −20 °C until analysis (approximately three–four weeks).

For the modified atmosphere treatment, heads were individually and hermetically enclosed in polyethylene bags (40 μm thick, 20 cm × 30 cm), and stored in darkness at 22 °C for 120 h. 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, in order to assure a homogeneous irradiation on florets, broccoli heads were placed upright on plastic trays under a bank of 4 UV-C lamps (TUV G30T8, 30W, Philips) (Costa et al., 2005b). The heads were irradiated at a distance of 30 cm to obtain a dose of 10 kJ m^{-2} s^{-1}. The flux intensity of lamps was measured with digital radiometer (Cole-Palmer Instrument Company, Vernon Hills, IL, USA). The same number of heads, not treated with UV-C, was utilized as controls. After treatment, the broccoli heads were also loosely covered with PVC and stored as described above. Samples were taken and processed as mentioned above.

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

2.5. Superficial colour measurement

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

2.6. Determination of chlorophyll contents

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 grams of total chlorophyll per kilogram of fresh tissue. All measurements were performed in quintuplicate.

2.7. Determination of lipoxygenase activity

Approximately one gram of frozen broccoli florets was homogenized with 10 mL of water at 4 °C and then centrifuged at 12 000 × g

for 15 min at the same temperature. Supernatants were utilized to determine lipoxygenase activity by spectrophotometry at 234 nm in the presence of linoleic acid (Zhuang et al., 1994). One unit of activity (UA) was defined as the increase of one unit of absorbance at 234 nm in a minute. All measurements were performed in quadruplicate.

2.8. Cloning of BoLOX1 from broccoli florets

Florets from broccoli 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 4 μg of total RNA was used as template for cDNA synthesis using MML-V reverse transcriptase (Promega) and random primers (hexamers). Resulting cDNA was employed as a template for PCR reaction and gene-specific primers. The primers for cloning *BoLOX1* were designed based on the *Arabidopsis thaliana* sequence (*At*1g55020) published in data base, forward: 5′-CGAAGAAGGTGAAAGGAACGGTG -3′, reverse: 5′-TGATCAAGAATGCTCCAGGGTAAC -3′. Amplification conditions consisted of an initial denaturating step of 94 °C for 4 min, followed by 36 cycles of 94 °C for 45 s, 68 °C for 45 s, 72 °C for 1 min. The final extension step was at 72 °C for 7 min. Amplified DNA fragment with approximated size of 294 bp was purified with Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare). Purified fragments were cloned using TOPO TA Cloning kit (Invitrogen). Clones obtained were sequenced from both directions with internal primers, M13-RV and T7-FW.

2.9. RNA extraction and real-time PCR

Broccoli florets 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). Approximately 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 template for two-step qPCR reactions using an Mx3005P real-time PCR system (Stratagene) and FastStart Universal SYBR Green Master (Roche). Actin (AF044573) was used as normaliser, forward: 5′- CCAGAGGTCTTGTTCCAGCCATC-3′ and reverse: 5′-GTTCCACCACTGAGCACAATGTTAC-3′. Primers specific to LOX1 were used: forward: 5′-TCTCCACGAATTTCTGGGAAACAAAG-3′, and reverse: 5′-AGTCCTCTGAGTGTGTAGCCTTTC -3′. Each measurement was performed in quintuplicate.

2.10. 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 BoLOX1

Previous work has been shown an increase in LOX activity during postharvest of broccoli (Page et al., 2001), which could be related to several processes associated to senescence. In *A. thaliana*, a gene named *AtLOX1* (At1g55020) has an increased expression during senescence of leaves (He et al., 2002). As *A. thaliana* belongs to *Brassica* family we designed specific primers utilizing this sequence as template to amplify a fragment from broccoli. The PCR reaction was successful and we obtained a fragment of 294 bp length, which was sequenced to confirm its identity and named *BoLOX1*.

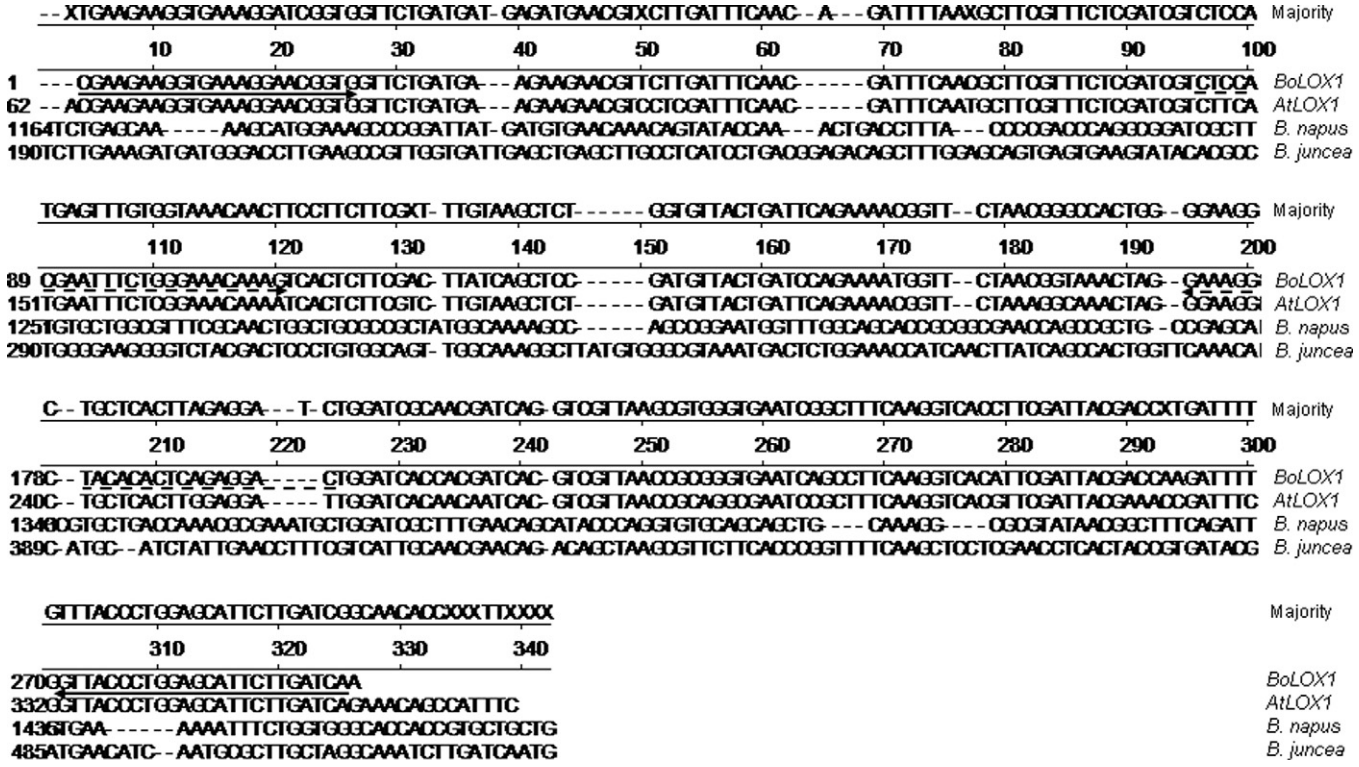

Fig. 1. Nucleotide sequence alignment of the fragment of *BoLOX1* cloned in this work with a fragment of *AtLOX1* from *Arabidopsis thaliana* (BT010358) and two other genes encoding lipoxygenases from *Brassica napus* (AY162142.1) and *Brassica juncea* (GU085237.1). The alignment was generated using the Clustal W method (Thompson et al., 1994). Solid arrows indicate sites chosen to clone the fragment of *BoLOX1* whereas dotted arrows indicate sites chosen for RT-qPCR experiments.

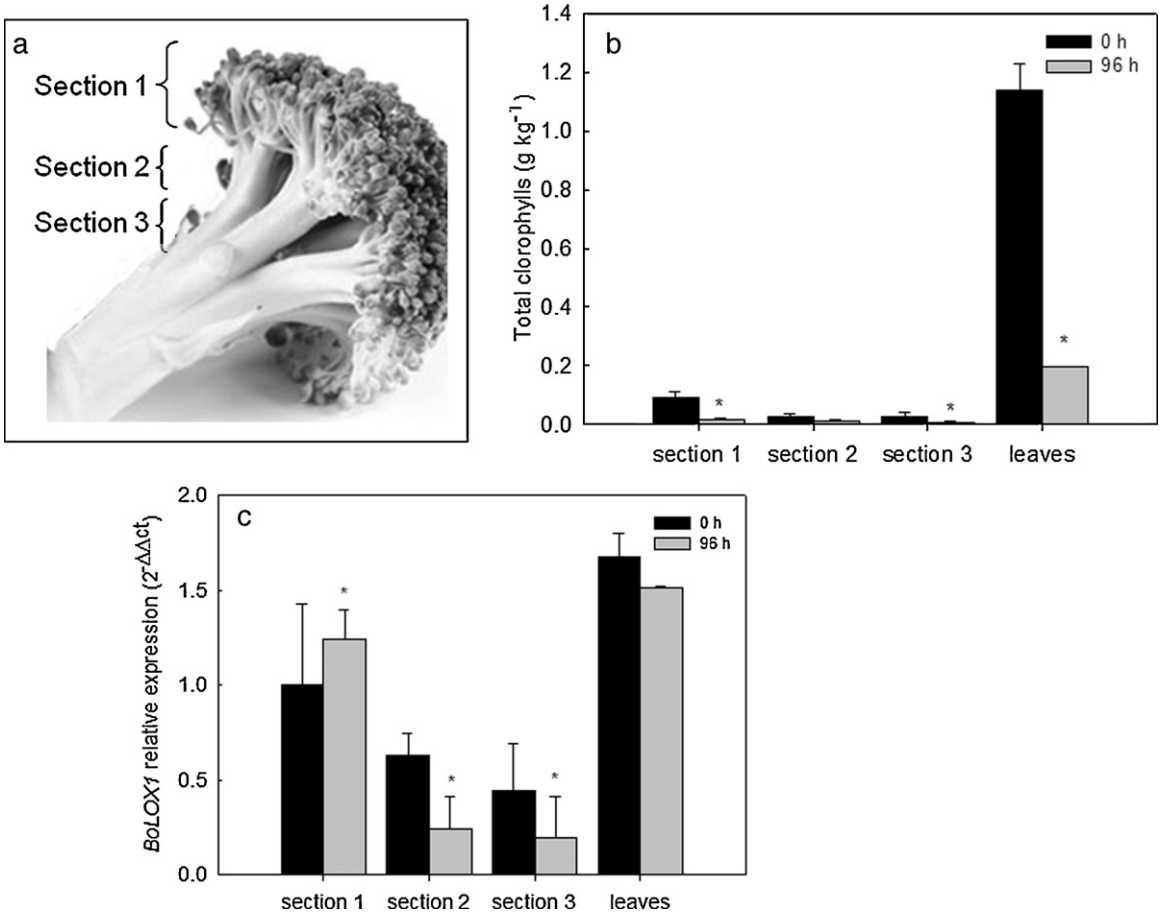

Fig. 2. Sectioning of broccoli florets (a). Total chlorophyll content in different sections of broccoli florets at day 0 and day 4 of induced senescence (b). Relative gene expression assessment of *BoLOX1* during senescence of different sections of broccoli florets (c). Asterisks show significant statistical differences between means obtained at day 0 and day 4. Means were obtained from 5 measurements and compared by the LSD test at a significance level of 0.05.

The cloned fragment has a 98% of identity with *Brassica napus* and 88% with *A. thaliana* (Fig. 1). To perform the spatial and temporal characterization of the expression of the corresponding gene we designed specific primers to amplify a fragment of 113 bp. In Fig. 1, it is showed an alignment of several of these genes, indicating the positions of primers employed for cloning and for RT-qPCR.

3.2. *BoLOX1* expression in different section of broccoli florets

In order to evaluate the expression of *BoLOX1* in different sections of broccoli florets we incubate inflorescences together with leaves at 22 °C in darkness for 4 d. Samples were segmented into Section 1 (inflorescence), Section 2 (small branchlets) and Section 3 (main floret stem) (Fig. 2a). The progress of senescence was followed by a decrease in chlorophyll content in the three sections of florets and leaves during the incubation. In Section 1 (inflorescence) was detected higher chlorophyll content compared with the other two sections (2 and 3). Leaves showed much higher chlorophyll contents than florets (Fig. 2b).

BoLOX1 expression was analyzed by RT-qPCR. We detected an increase in the expression of *BoLOX1* in Section 1 after 96 h. In Sections 2 and 3 the expression was lower than that of Section 1 and decreased after the same time. In leaves, it was detected a high expression in pre-senescent tissues which slightly decreased after the end of the incubation (Fig. 2c).

3.3. *BoLOX1* expression during postharvest senescence

As the main change in *BoLOX1* expression during senescence was detected in the inflorescences, we decided to analyze the expression with more detail in this tissue. To do that, florets were incubated as described previously but samples were taken at 0 h and 24, 48, 72 and 120 h of treatment. Chlorophyll content was measured to follow the senescence. We observed that chlorophyll levels remained without changes until 48 h and then diminished significantly (Fig. 3a).

The *BoLOX1* expression showed a decrease of about 50% after 24 h and then increased its expression until 120 h, reaching the double of expression compared with initial values (Fig. 3b).

3.4. Effects of hormone treatment on BoLOX1 expression and lipoxygenase activity

We analyzed the effect of treatments with cytokinins (BAP) and ethylene (ethephon) on *BoLOX1* expression and lipoxygenase activity during senescence of broccoli florets. In controls, the Hue angle decreased slightly after 48 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 colour until the end of treatment. On the contrary, ethephon treatment caused a larger decrease in hue (Table 1). The chlorophyll content also decreased during storage (Fig. 4a). Samples treated with ethephon showed lower chlorophyll content in relation to controls, while samples treated with BAP showed lower chlorophyll degradation. In the case

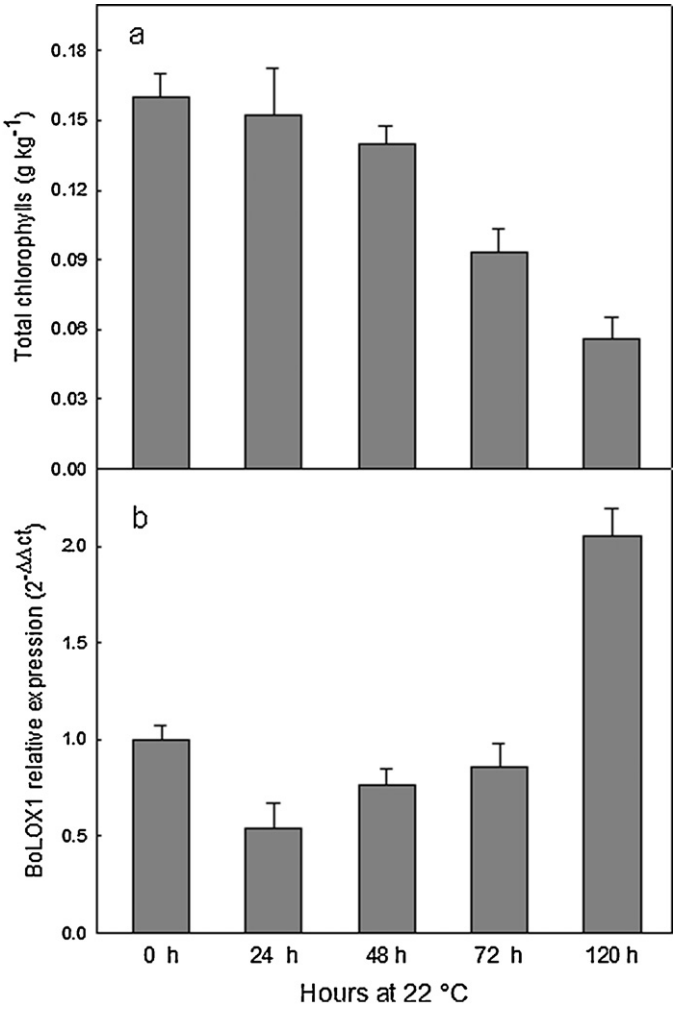

Fig. 3. Chlorophyll content (a) and relative *BoLOX1* expression (b) of broccoli heads during 5 d of dark induced senescence at 22 °C.

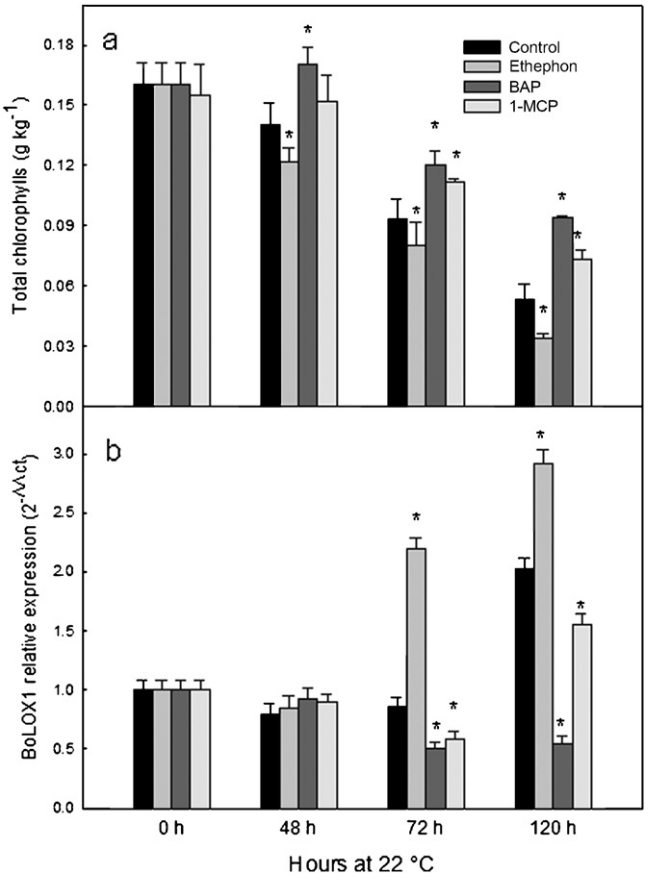

Fig. 4. Chlorophyll content (a) and relative *BoLOX1* expression (b) of hormone-treated broccoli heads during 5 d of induced senescence at 22 °C. Treatments with ethephon and BAP were done by immersing the heads in solutions containing the hormones for 10 min. Treatment with 1-MCP was done by putting the heads in a hermetic container for 16 h. Asterisks show significant statistical differences between means of each treated samples and controls at the same time. Means were obtained from 5 measurements and compared by the LSD test at a significance level of 0.05.

of treatment with 1-MCP, a higher hue value in relation to controls (Table 1) and lower chlorophyll degradation after 72 and 120 h of storage (Fig. 4a) was detected.

The *BoLOX1* expression increased after 120 h in controls. The treatment with ethephon caused an accelerated increase of the expression at 72 h, which maintained higher than controls after 120 h. In contrast, BAP treatment caused a complete inhibition of the increase of the expression after 120 h and values maintained similar to initials (Fig. 4b). Treatment with 1-MCP slightly reduced the expression of *BoLOX1* after 72 h and caused a lesser increase in relation to controls after 120 h.

Total lipoxygenase activity increased in control samples after 72 h of incubation and then slightly decreased (Fig. 7a). Samples treated with ethephon showed a larger increase of activity after 72 h, reaching values that doubled those of controls. After 120 h, a decrease of activity was detected although values were still higher than controls. Differently, BAP treatment caused an inhibition of the increase of lipoxygenase activity after 72 h and only after 120 h the activity increased.

3.5. Effect of postharvest physical treatments on BoLOX1 expression and lipoxygenase activity

We performed treatments with hot air and modified in order to evaluate their effect on the expression of *BoLOX1* and lipoxygenase activity. In controls, the hue values remained unchanged until 72 h and then decreased while samples treated with hot air or modified atmosphere maintained higher hue values until the end of experiment (Table 1). The levels of total chlorophyll decreased in control

Table 1
Hue values of florets from broccoli heads exposed to different hormones and physical treatment and then stored at 22 °C for 0 h, 72 h and 120 h.

	Control	Ethephon	1-MCP	BAP	Modified atmosphere	Heat treatment	Visible light	UV-C
0 h	125.75 ± 2.89	125.75 ± 2.89	125.35 ± 2.62	125.75 ± 2.89	125.75 ± 2.89	125.75 ± 2.89	125.75 ± 2.89	125.75 ± 2.89
72 h	123.91 ± 3.86	120.00 ± 3.84*	125.99 ± 2.05*	127.37 ± 2.65*	125.40 ± 2.95*	123.93 ± 3.15*	126.53 ± 1.73*	121.91 ± 1.65
120 h	102.63 ± 8.09	98.79 ± 2.44*	111.10 ± 3.77*	127.06 ± 2.51*	116.89 ± 6.95*	121.71 ± 2.17*	123.20 ± 6.93*	110.49 ± 3.87*

Asterisks show significant statistical differences between means of each treated samples and controls at the same time. Means were obtained from 200 measurements and compared by the LSD test at a significance level of 0.05. Treatments with ethephon and BAP were done by immersing the heads in solutions containing the hormones for 10 min. Treatment with 1-MCP was done by putting the heads in a hermetic container for 16 h at 22 °C. For the modified atmosphere treatment, samples were hermetically enclosed in polyethylene bag. For hot air treatment heads were maintained in hot air at 42 °C for 3 h. For the visible light treatments, heads were placed up right in plastic bowls exposed to a dose of 12 μmol m^{-2} s^{-1}. For the UV-C treatment, heads were placed up right on plastic trays and exposed to a dose of 10 kJ m^{-2} s^{-1}.

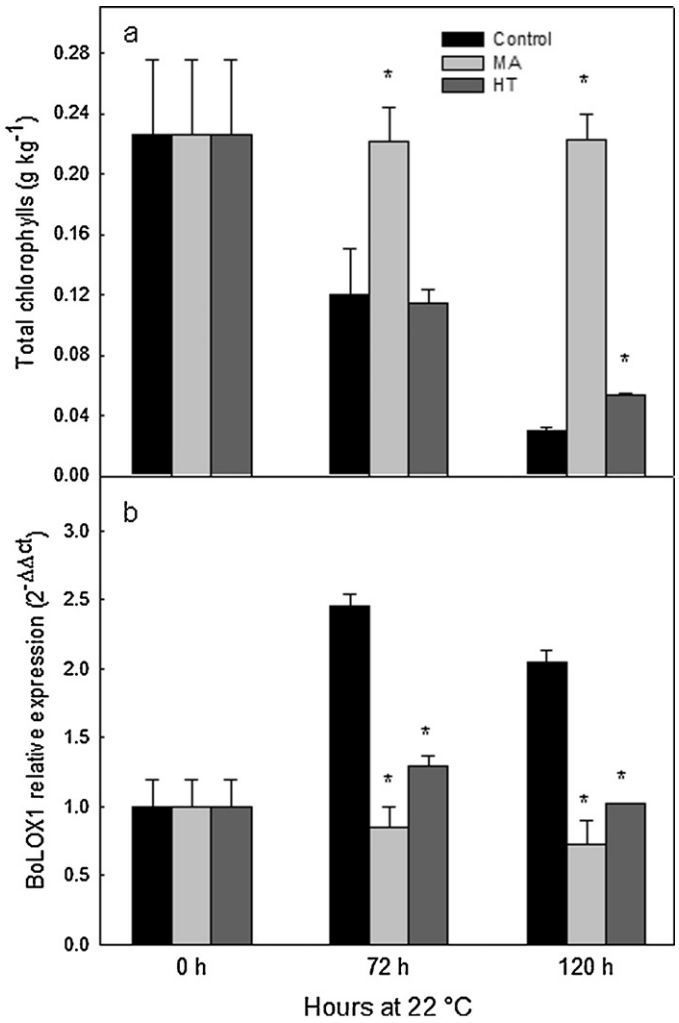

Fig. 5. Chlorophyll content (a) and relative *BoLOX1* expression (b) of broccoli florets subjected to heat or modified atmosphere treatments during 5 d of induced senescence at 22 °C. For the modified atmosphere treatment, samples were hermetically enclosed in polyethylene bag. For hot air treatment heads were maintained in hot air at 42 °C for 3 h. Asterisks show significant statistical differences between means of each treated samples and controls at the same time. Means were obtained from 5 measurements and compared by the LSD test at a significance level of 0.05.

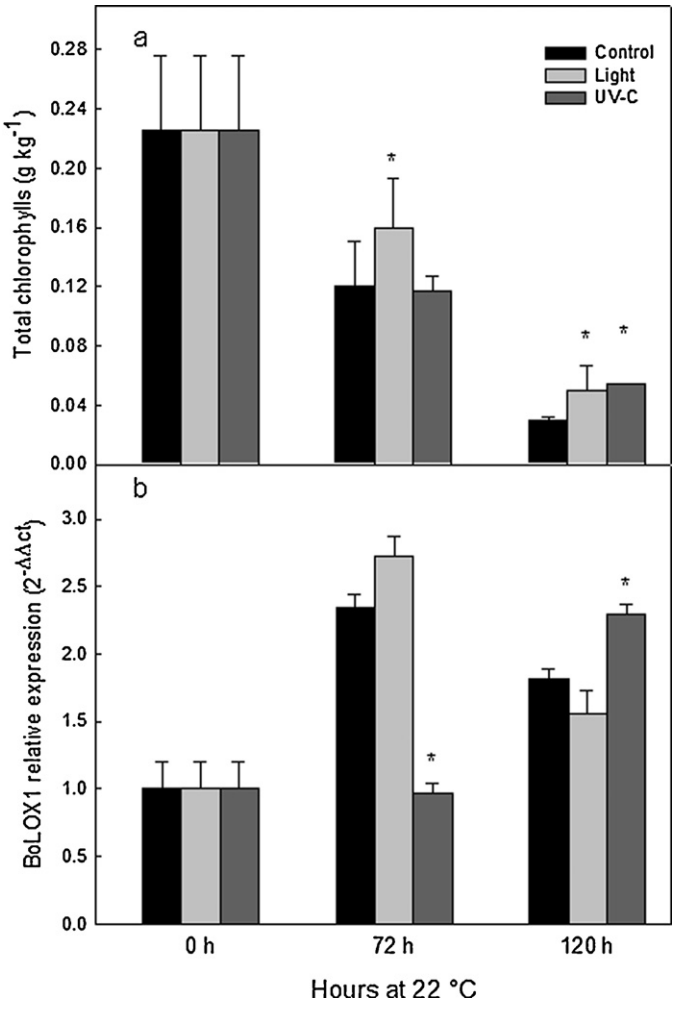

Fig. 6. Chlorophyll content (a) and relative *BoLOX1* expression (b) of broccoli florets subjected to visible light or UV-C treatments during 5 d of induced senescence at 22 °C. For the visible light treatments, heads were placed up right in plastic bowls exposed to a dose of 12 μmol m^{-2} s^{-1}. For the UV-C treatment, heads were placed up right on plastic trays and exposed to a dose of 10 kJ m^{-2} s^{-1}. Asterisks show significant statistical differences between means of each treated samples and controls at the same time. Means were obtained from 5 measurements and compared by the LSD test at a significance level of 0.05.

samples. In samples treated with modified atmosphere, the levels of chlorophyll were maintained throughout the experiment. Level of chlorophylls in hot air treated samples also showed a decrease but values were higher than controls after 120 h (Fig. 5a).

The expression of *BoLOX1* in controls increased after 72 h and then remained without significant changes until the end of the treatment. Both physical treatments inhibited an increase in the expression of this gene but the effect of modified atmosphere was more marked (Fig. 5b).

A similar behavior of lipoxygenase activity was detected in controls of physical treatment experiments (Fig. 7b). In samples stored in modified atmosphere, total lipoxygenase activity showed an important increase after 72 h and then decreased to values lower than controls, while samples treated with heat showed a decrease of lipoxygenase activity only after 72 h.

We also perform treatments with different kinds of radiations to evaluate their effects on *BoLOX1* expression. Previous works have demonstrated that treatment with UV-C (Costa et al., 2005b) or visible light (Büchert et al., 2011b) can delay broccoli senescence. Hue values of light and UV-C treated samples were higher than controls (Table 1). The levels of chlorophylls decreased during the incubation in control and treated samples. In light treated samples content of chlorophylls were higher than in the controls, while the UV-C treatment has higher contents than controls only after 120 h (Fig. 6a).

As in previous experiments, the expression of *BoLOX1* in control samples increased at 72 h and then slightly decreased. The light treatment did not affect the expression of *BoLOX1*, which showed a similar pattern of expression to controls. In the case of UV-C the treatment avoided the increase of expression detected at 72 h but it increased after 120 h reaching values similar to controls (Fig. 6b).

In relation to lipoxygenase activity, samples treated with visible light showed an important increase after 72 h that decreased to make equal to controls after 120 h (Fig. 7c). Finally, heads irradiated with UV-C presented lipoxygenase activities similar to those of controls during the storage.

4. Discussion

One of the catabolic metabolisms associated to senescence is membrane lipid degradation, a process that contributes to

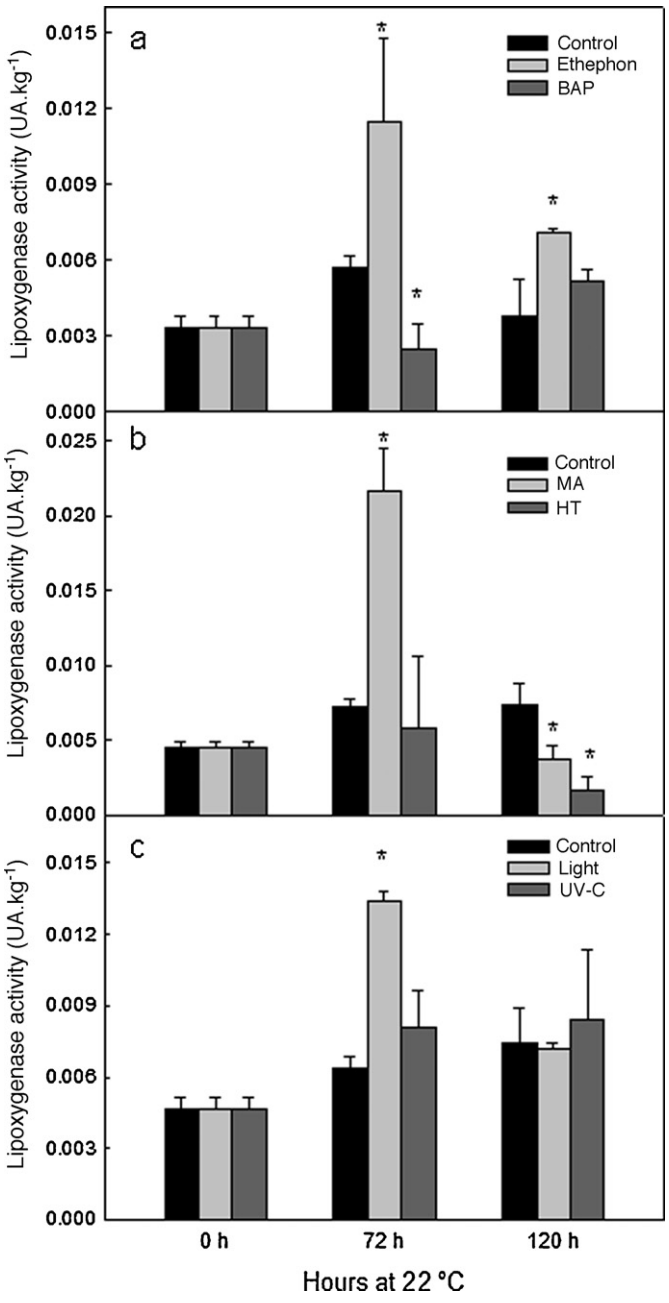

Fig. 7. Changes in lipoxygenase activity of broccoli heads during 5 d of induced senescence at 22 °C. Hormone (ethephon and BAP) and physical treatments (modified atmosphere, heat, visible light and UV-C) were done as described above. Asterisks show significant statistical differences between means of each treated samples and controls at the same time. Means were obtained from 4 measurements and compared by the LSD test at a significance level of 0.05.

postharvest deterioration of fruits and vegetables. Lipid degradation can be carried out by an enzymatic oxidation by LOX or by non-enzymatic oxidation by reactive oxygen species. In pepper, for example, it was suggested that LOX catalyzed oxidation of membrane lipids can cause such a damage conducting to loss of membrane integrity and accelerate water loss (Maalekuu et al., 2006). In addition, LOX is the first enzyme of the metabolic pathway of biosynthesis of jasmonic acid, which in turn is involved in promoting senescence (He et al., 2002).

In broccoli, a decrease in the content of fatty acid (Zhuang et al., 1997) and an increase in the level of lipid peroxidation (Zhuang et al., 1995) have been described during postharvest senescence. For these reasons, analysis and characterization of genes and enzymes involved in lipid catabolism and jasmonic acid biosynthesis becomes interesting in this system. In this work we cloned a fragment of gene encoding a lipoxygenase belonging to *B. oleracea* that showed similarities to previously reported sequences of LOX genes. The cloned sequence was highly similar to a previous gene reported in Arabidopsis (*AtLOX1*) (Melan et al., 1993).

Studies about changes in LOX activity during broccoli senescence have shown contradictory results. Zhuang et al. (1997) detected a decrease in LOX activity over time, while Lan Eum et al. (2009) found a slight increase after 2 d at 20 °C. Differently, Page et al. (2001) reported an increase of seven times in the activity after 4 d of storage at 20 °C. We detected an increase of activity after 3 d and then a decrease at the end of experiment. Differences in the changes of LOX activity among studies could be due to differences in senescence rates probably caused by differences in experimental conditions or in cultivar varieties utilized.

LOX genes belong to a family that is represented by several members in most species and their expression is usually differentially regulated depending on the physiological process. For example, *Vitis vinifera* have been described in 18 individual members of the LOX family, but only two have an enhanced expression during ripening (Podolyan et al., 2010). Similarly, six genes of LOX were cloned and characterized in kiwi and only two of them are related to ripening (Zhang et al., 2006). In Arabidopsis, six genes have been described (*AtLOX1*, *AtLOX2*, *AtLOX3*, *AtLOX4*, *AtLOX5* and *AtLOX6*) but only three of them seem to be involved in leaf senescence (He et al., 2002; Melan et al., 1993; Schaller et al., 2004).

We found that the expression of *BoLOX1* increased during postharvest senescence. The higher expression was detected at the end of the experiment when heads were completely yellow. The expression of some LOX encoding genes is enhanced by tissue damage (Bell et al., 1995; Podolyan et al., 2010). In this sense, severe damage that takes place during the last steps of senescence may enhance the expression of *BoLOX1*.

In a previous work it was shown that postharvest senescence of broccoli can be regulated positively by ethylene and negatively by cytokinins (Costa et al., 2004). These hormones can also regulate the expression of senescence related genes such as *BoPPH*, a gene related to chlorophyll catabolism (Büchert et al., 2011a) or *BoACO1* and *BoACO2*, which are involved in ethylene biosynthesis (Chen et al., 2008). We found that ethylene greatly enhanced *BoLOX1* transcript levels and lipoxygenase activity, while BAP inhibited the peak of expression detected during senescence and delayed the increase of activity. According to this, treatment with 1-MCP, a blocking agent of ethylene action, delayed the increase of *BoLOX1* during senescence. In kiwi, treatment with ethylene enhanced transcript accumulation of LOX genes was associated to ripening (Zhang et al., 2006).

In broccoli, several studies have focused on the use of postharvest treatments to reduce postharvest senescence (Yamauchi et al., 1997; Costa et al., 2006; Ku and Wills, 1999). The utilization of envelopes, such as polyethylene bags that induce modified atmospheres, is very useful for delaying senescence in broccoli (Eason et al., 2007) during storage at 20 °C. To our knowledge, this is the first report that showed the effect of modified atmosphere on the expression of a LOX encoding gene. We found an important inhibition of the peak of *BoLOX1* expression in samples stored under modified atmospheres.

It has been shown that heat treatment can reduce senescence by delaying associated catabolism (Costa et al., 2005a; Funamoto et al., 2002). We found that heat treatment caused an important inhibition of the increase of *BoLOX1* expression after 72 h and 120 h. In peaches, a heat treatment of 38 °C during 12 h causes a reduction in LOX activity during storage (Cao et al., 2010). Similarly, a heat treatment of 42 °C for 15 min diminishes the peak of LOX activity

during storage of the banana cultivar "Gros Michel" (Promyou et al., 2008).

In the case of UV-C, this treatment also provokes a delay in broccoli senescence (Costa et al., 2005b). In this work, UV-C treated samples showed a lower expression of *BoLOX1* at 72 h but a similar expression after 120 h in relation to controls. Low doses of UV-C can cause a transitory inhibition of gene expression, which recover when the tissue is returned to non-stressing conditions (Pombo et al., 2009). In the present work, treatments with UV-C may have caused a delay in the increase of *BoLOX1* expression at 72 h, which recovered at 120 h.

Recently, it has been shown that a treatment with visible light can delay postharvest senescence in broccoli heads stored at 22 °C (Büchert et al., 2011b). We applied a similar treatment and obtained an important maintenance of green colour, but *BoLOX1* expression showed a similar behavior in samples treated with light in relation to controls.

Changes in lipoxygenase activity in samples subjected to physical treatments did not show the same patterns as those detected in *BoLOX1* expression. Treatments with modified atmospheres, heat and UV-C inhibited or delayed *BoLOX1* expression, but they did not cause the same effect on lipoxygenase activity. Moreover, modified atmospheres and visible light induced a marked increase in the activity. In this sense, Zhuang et al. (1995) also found a higher lipoxygenase activity in samples stored under modified atmosphere packaging after 72 h of storage at 5 °C. As mentioned previously, plants have several LOX genes and the activity results from the combined expression of these genes. It could occur that treatments like modified atmospheres and visible light greatly enhance the expression of other LOX genes rather than *BoLOX1* and thus contribute to the increase of total activity.

In conclusion, we cloned a fragment of a gene encoding a putative LOX (*BoLOX1*) from broccoli, a horticultural crop with enhanced postharvest senescence. We characterized the expression of the gene in different parts of the inflorescence during postharvest senescence and found a close correlation between this process and expression of *BoLOX1*. The expression was also analyzed in samples treated with different hormones and postharvest physical treatments, reaffirming the relationship mentioned previously.

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