

Effect of electrostatic atomization on ascorbate metabolism in postharvest broccoli

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3 **broccoli**

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Abstract

In the present study, the effects of electrostatic atomization on ascorbate (AsA) metabolism were studied and the possible molecular mechanisms were discussed in broccoli (*Brassica oleracea* L. var. *italica*). With the treatment of electrostatic atomization, the yellowing process was delayed, and the ethylene production and respiration rate were significantly suppressed in broccoli after harvest. In the meanwhile, the AsA content declined rapidly to a lower level in the control after harvest, and the reduction of AsA was suppressed by the treatment with electrostatic atomization during the storage period. Additionally, the modulation of the AsA reduction by electrostatic atomization was highly regulated at the transcription level. The up-regulation of the AsA biosynthetic genes (*BO-VTC1*, *BO-VTC2* and *BO-GLDH*), and AsA regeneration genes (*BO-MDAR1*, *BO-MDAR2* and *BO-DHAR*) led to the suppression of AsA reduction in the electrostatic atomization treated broccoli after harvest. These results indicated that electrostatic atomization treatment might be a new effective approach for delaying the senescence of broccoli.

Key words: Ascorbate, Broccoli, Electrostatic atomization, Real-time PCR, Senescence

1. Introduction

Ascorbate (AsA), also known as vitamin C, is one of the most abundant antioxidants in plants (Davey et al., 2000; Foyer and Noctor, 2005). It is essential for plant growth and development, and plays an important role in resistance to environmental stresses and the commencement of senescence (Davey et al., 2000; Alhagdow et al., 2007; Ioannidi et al., 2009). In addition, AsA acts as a cofactor of many dioxygenases that involve in the key steps of cell metabolism (Smirnoff et al., 2001). In the recent years, several AsA biosynthetic pathways have been described, and among them the L-galactose pathway was identified to be a major AsA biosynthetic route in plants (Conklin et al., 2000; Dowdle et al., 2007; Linster and Clarke, 2008). As shown in Fig. 1, D-mannose-1-phosphate is converted into GDP-D-mannose by GDP-D-mannose pyrophosphorylase (VTC1). GDP-D-mannose is further converted into GDP-L-galactose via the action of GDP-D-mannose 3',5'-epimerase. Free L-galactose is released from GDP-L-galactose by the GDP-L-galactose phosphorylase (VTC2) and L-galactose-1-phosphate phosphatase, and then is oxidized by L-galactose dehydrogenase to form L-galactono-1,4-lactone. L-Galactono-1,4-lactone is oxidized to AsA by L-galactono-1,4-lactone dehydrogenase (GLDH). AsA is not a stable metabolic product and can be oxidized by ascorbate peroxidase (APX) to produce monodehydroascorbate (MDA), which can be reduced enzymatically to AsA by NADPH-dependent MDA reductase (MDAR), or dismutate spontaneously to dehydroascorbate (DHA). DHA is reduced to AsA enzymatically in a reaction mediated by DHA reductase (DHAR), using glutathione as an electron donor. The

resulting oxidized glutathione is then converted back to the reduced form (GSH) by an NADPH-dependent glutathione reductase (Nishikawa et al., 2003a). In higher plants, AsA metabolism is complicated, which is co-regulated by the synthesis, oxidation and recycling processes. The enzymes related to the AsA metabolism exist as isoenzymes distributed in distinct cellular organelles: chloroplasts, plastids, mitochondria, and peroxisomes (Mittova et al., 2000).

As humans can not synthesize or store the AsA in the body, the fruits and vegetables are the primary sources of AsA intake for humans. Broccoli (*Brassica oleracea* L. var. *italica*) is a popular green vegetable in the world. Epidemiological studies have shown that the consumption of broccoli might decrease the risk of certain cancers (Day et al., 1994; Nath et al., 2011). The protective effects might be attributed to the higher content of antioxidants in broccoli, such as AsA, flavonoids and carotenoids. However, when broccoli heads are harvested, their florets are still immature. The immature organs are very sensitive to stresses, which makes them senesce rapidly after harvest. During the senescence process, the AsA content decreased rapidly in the florets of broccoli (Nishikawa et al., 2003a). So far, extensive methods have been investigated to delay the post-harvest senescence of broccoli, such as the use of a modified atmosphere (MA) or controlled atmosphere (CA), different types of packaging, and treatment with chemicals and cytokinins (Hansen et al., 2001; Toivonen and DeEll, 2001; Jia et al., 2009; Nath et al., 2011). In the previous study, we found that the application of 1-methylcyclopropene (1-MCP), which is an effective inhibitor of ethylene action, was effective to delay yellowing and decay, alleviate certain ethylene-induced postharvest

physiological disorders, and extend the shelf-life of broccoli (Ma et al., 2009). Furthermore, the treatment of 1-MCP could delay the reduction of AsA in broccoli after harvest, and the modulation of the AsA metabolism contributed to the beneficial effects of 1-MCP on the senescence in broccoli (Ma et al., 2010).

Electrostatic atomization, which is also called as electrospray or electrohydrodynamic atomization, is a method typically used to produce fine liquid droplets with diameters between ten and several hundred micrometers and a relatively narrow size distribution (Mori and Fukumoto, 2002). As shown in Fig. 2, the electrostatic atomization device mainly contains four parts: cooling fin, Peltier element, discharge electrode and ground electrode (Kobayashi et al., 2007; Yamauchi et al., 2007). Water is dewed by Peltier element in the discharge electrode. When applying a voltage between the discharge electrode and ground electrode, the liquid droplets were formed by dewed water at the tip of the discharge electrode. Compared with other atomization techniques, the electrostatic atomization has some advantages such as: easy handling of droplets by applying an electric field and avoiding coalescence of droplets due to electric charge of the same polarity on the droplets, a narrow size distribution of generated droplets (Watanabe et al., 2003). It has been reported that electrostatic atomization treatment could suppress the growth of viruses and pathogenic bacteria (Asano et al., 2010). To date, however, the application of electrostatic atomization in the post-harvest preservation of fruits and vegetables is a new area in research (Nakada et al., 2008). In the present study, the effects of electrostatic atomization on the senescence of broccoli were investigated. In the

meanwhile, the roles of electrostatic atomization in regulating the AsA metabolism in broccoli after harvest were discussed. The results arising from this study will help to provide a new effective method to delay the senescence of broccoli after harvest.

2. Materials and Methods

2.1. Plant materials and treatments

‘Haitsu’ broccoli (*Brassica oleracea* L. var. *italica*) plants were grown at the Fujieda Farm of Shizuoka University, Shizuoka, Japan. Mature broccoli heads of uniform size, shape, and maturity were selected and continuously treated with electrostatic atomization (Panasonic, Japan) in acrylic chambers (60 cm × 43 cm × 34 cm). The control heads were held in the chambers without electrostatic atomization device. In the present study, ~5 kV voltage was applied between the discharge electrode and ground electrode, and liquid droplets with about 20 nm diameter were formed by dewed water at the tip of the discharge electrode. All treatments were conducted at 20 °C under highly humidified conditions (RH > 95%). Florets were excised from the heads with a single-edged razor every day after harvest, and three replicates of three heads of broccoli were taken at each sampling time. The excised florets were immediately frozen in liquid nitrogen except for the samples for ethylene production and respiration rate analyses, and stored at -80 °C until used.

2.2. Assessment of broccoli yellowing

The colour of florets in broccoli was scored by a visual assessment of changes from green to yellow. A rating scale of senescence from 5 to 0 was adopted: 5, all green; 4,

20% yellowing; 3, 40% yellowing; 2, 60% yellowing; and 1, 80% yellowing. Intermediate numbers were assigned where appropriate according to the yellowing rate.

2.3. Measurements of ethylene production and respiration rate

A 1-g sample of florets was placed into a 15-mL vial, the vial was sealed using a silicon rubber cap, and sample was incubated for 30 min at 20 °C. The headspace gas in the vial was sampled using a 1-mL plastic hypodermic syringe and injected into a gas chromatograph (Hitachi 163) for ethylene or into a gas chromatograph (Hitachi 164) for carbon dioxide. The rate of ethylene production was expressed as nl ethylene per h per g FW. The respiration rate was expressed as mg CO₂ per h per g FW.

2.4. Extraction and assays of AsA and DHA

The contents of AsA and DHA were assayed by HPLC. Each frozen sample was homogenized using a mortar and pestle in 10 volumes of extractant solution (3% metaphosphoric acid and 8% acetic acid). The homogenate was centrifuged at 14,000× g for 20 min, and then the supernatant was filtered through Miracloth (Calbiochem). The pH of the filtrate was adjusted by adding an equal volume of 0.2 M potassium-phosphate buffer (pH 7.5). Total amount of AsA and DHA was assayed by adding 0.5 ml of 6 mM dithiothreitol (DTT) to 0.1 ml of aliquot of filtrate and incubated in the dark at 30 °C for 15 min. After the sample was filtered through a 0.22-µm cellulose acetate filter (Advantec), a 20 µl aliquot was injected onto a J'sphere ODS-M80 column (YMC) attached to a LC-10AD pump (Shimadzu). The column kept at 20 °C was eluted with 1.5% ammonium dihydrogen phosphate (pH 3.8) at a flow rate

of 1.0 ml min⁻¹. The absorbance at 245 nm (retention time 2.6 min) was monitored using an SPD-10A spectrophotometric detector (Shimadzu) attached to a chart recorder (C-R6A, Shimadzu). Peaks were converted to concentrations by using the dilution of stock ascorbic to construct a standard curve. AsA content was determined in a similar manner without the addition of DTT. DHA content was calculated by subtraction the AsA value from the total amount.

2.5. Total RNA extraction and real-time quantitative RT-PCR

Total RNA was extracted from the florets of broccoli after harvest according to the method described by Kato et al. (2000). The total RNA was cleaned up using the RNeasy Mini Kit (Qiagen, Hilden, Germany) with on-column DNase digestion. The reverse transcription reactions were performed with 2 µg of purified RNA and a random hexamer at 37 °C for 60 min using TaqMan Reverse Transcription Reagents (Applied Biosystems).

In broccoli, the information on the *BO-VTC1* and *BO-VTC2* sequences were not reported previously. In the present study, the degenerate PCR primers for *BO-VTC1* and *BO-VTC2* were designed according to the common sequences of apple, tobacco and Arabidopsis. Primer pairs used for amplification were as follows: *BO-VTC1* (Forward: ATCACNTGYTCNCAAGARAC; Reverse: ACRTCNGGTCCDATCAARCA); *BO-VTC2* (Forward: TATGGNCATGTNCTNYTGAT; Reverse: ACCATRTGNCCACTDATYTC). The amplified cDNAs were cloned with a TA Cloning Kit (Invitrogen, San Diego). The sequences were determined using the Taq Dye Primer Cycle Sequencing Kit (Perkin Elmer Applied Biosystems,

Foster City, CA).

TaqMan MGB probes and sets of primers for *BO-VTC1*, *BO-VTC2*, *BO-GLDH*, *BO-APX1*, *BO-APX2*, *BO-sAPX*, *BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR* were designed with the Primer Express software (Applied Biosystems; Table 1). For endogenous control, the TaqMan Ribosomal RNA Control Reagents VIC Probe (Applied Biosystems) was used. TaqMan real-time PCR was carried out with the TaqMan Universal PCR Master Mix (Applied Biosystems) using StepOnePlus™ Real-Time PCR System (Applied Biosystems) according to the manufacture's instructions. Each reaction contained 900 nM primers, a 250 nM TaqMan MGB Probe, and template cDNA. The thermal cycling conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The levels of gene expression were analyzed with StepOnePlus™ Real-Time PCR System Software (Applied Biosystems) and normalized with the results of 18S ribosomal RNA. Real-time quantitative RT-PCR was performed in three replicates for each sample.

2.6. Statistical analysis

All values are shown as the mean \pm SE for three replicates. The data were analyzed, and Student's *t*-test was used to compare the treatment means at $P < 0.05$.

3. Results

3.1 Effect of electrostatic atomization on visual quality, ethylene production and respiration rate

The transition of colour from green to yellow is a direct index of senescence of

broccoli. As shown in Fig. 3A, the florets began yellowing rapidly from the third day after harvest. Compared with the control, the yellowing process was delayed by the treatment with electrostatic atomization. On the third day, the florets displayed obvious yellowing in the control, but in the treatment of electrostatic atomization most of the florets still kept green (Fig. 3A).

Ethylene production decreased slightly on the first day, and then increased rapidly in the control. With the treatment of electrostatic atomization, ethylene production was 15% and 40% lower than that of the control on the third and fourth days, respectively (Fig. 3B). In the control, respiration rate decreased after harvest, and then increased with a peak on the third day. Compared with the control, the respiration rate was decreased by the treatment of electrostatic atomization, which was 10% and 14% lower than that of the control on the third and fourth days, respectively (Fig. 3C).

3.2 Effect of electrostatic atomization on AsA content

In the control, the AsA content decreased rapidly after harvest. Treatment with electrostatic atomization clearly slowed the ascorbate reduction after harvest (Fig. 4A). The AsA content in the electrostatic atomization treatment was about 1.2-1.8 times of that in the control during the whole storage period. The content of DHA was relatively low in broccoli, which was less than 10% of total ascorbate throughout the experimental period. The proportion of DHA varied little in both experimental treatments (Fig. 4B).

3.3 Effect of electrostatic atomization on expression of genes related to AsA metabolism

To further elucidate how the AsA metabolism was regulated by electrostatic atomization treatment, the changes in the expression of AsA biosynthetic genes (*BO-VTC1*, *BO-VTC2* and *BO-GLDH*), AsA oxidation genes (*BO-APX1*, *BO-APX2* and *BO-sAPX*), and AsA regeneration genes (*BO-MDAR1*, *BO-MDAR2* and *BO-DHAR*) were addressed in the present study (Fig. 5). In the control, the expression of *BO-VTC1* and *BO-VTC2* decreased slightly after harvest, and then increased with a peak on the third and second days, respectively. The expression of the mitochondrial gene (*BO-GLDH*) decreased significantly to a low level on the first day, and then kept almost unchanged in the control. With the treatment of electrostatic atomization, the expression levels of *BO-VTC1*, *BO-VTC2* and *BO-GLDH* were higher than those of the control during the storage period.

The expression of *BO-APX1*, *BO-APX2* and *BO-sAPX*, decreased slightly after harvest, and then increased significantly with a peak on the third day in the control. With the treatment of electrostatic atomization, the expression peak of *BO-APX1*, which appeared on the second day, was one day earlier than that of the control. In contrast, the expression of *BO-APX2* and *BO-sAPX* was not affected by the treatment of electrostatic atomization.

In the control, the expression of *BO-MDAR1* and *BO-MDAR2* decreased rapidly on the first day after harvest, and then increased slightly. The expression of *BO-DHAR* decreased gradually after harvest in the control. In the treatment of electrostatic atomization, the expression levels of *BO-MDAR1*, *BO-MDAR2* and *BO-DHAR* were higher than those of the control during the storage period.

4. Discussion

Broccoli is a highly perishable horticultural crop with a short shelf life in air at 20 °C (Suzuki et al., 2004). After harvest, the florets of broccoli senesced rapidly, and the expression of many genes related to broccoli senescence was induced within 24 h after harvest (Hasperué et al., 2011). The green colour of broccoli is an important commercial quality index (Jia et al., 2009; Yuan et al., 2010; Nath et al., 2011). During the post-harvest period, as any appearance of yellowing will terminate the shelf life of broccoli, it is crucial to prevent broccoli florets from yellowing after harvest. Recently, some techniques have been applied to maintain the green colour of broccoli florets (Aiamla-or et al., 2009; 2010; Nath et al., 2011). In addition, broccoli is a typical climacteric species, senescing rapidly after the appearance of ethylene climacteric peak (Makhlouf et al. 1989; Hyodo et al., 1994; King and Morris et al., 1994; Kasai et al., 1996). Thus, treatments that reduce ethylene production or inhibit its perception might be effective in delaying the senescence process in broccoli (King and Morris et al., 1994; Kasai et al., 1996). In the previous studies, we found that the delay of ethylene synthesis by 1-MCP, which is an effective inhibitor of ethylene action, led to improve the shelf life of broccoli (Ma et al., 2009; 2010). In the present study, the effects of electrostatic atomization on senescence of broccoli were investigated. The results showed that the yellowing process was delayed one day by the treatment of electrostatic atomization (Fig. 3A). Additionally, the ethylene production and respiration rate were suppressed by the treatment of electrostatic

atomization on the third and fourth days (Figs. 3B and C). These results suggested that electrostatic atomization treatment was effective to delay the senescence of broccoli after harvest. As broccoli is a highly perishable horticultural crop with a short shelf life in air at room temperature, the extension of the shelf life and maintaining the visual quality of broccoli by an extra day after harvest in the electrostatic atomization treatment will contribute to reduce the economic loss for the growers and provide much fresher vegetables for the consumers.

In the recent year, the researches into the improvement of AsA level in plants is becoming of great importance and has gained more attention because of its healthy beneficial effects (Chen et al., 2003; Ioannidi et al., 2009; Gergoff et al., 2010; Ma et al., 2010, 2011; Eltelib et al., 2011). Fresh broccoli is rich in AsA, however, during the storage period AsA is sensitive to destruction and its content in the florets of broccoli decreased rapidly to a low level after harvest (Nishikawa et al., 2003a). The losses of AsA could be suppressed by postharvest handling, such as heat, ethanol vapor and 1-MCP treatments (Shigenaga et al., 2005; Mori et al., 2009; Ma et al., 2009, 2010). In the present study, the results showed that the treatment with electrostatic atomization slowed the AsA reduction during the senescence process. The AsA content in the electrostatic atomization treatment was higher than that of the control during the whole storage period (Fig. 4). In the electrostatic atomization device, simultaneous formation of radicals in the liquid droplets, such as superoxide and hydroxyl radical were detected by using electron spin resonance (Yamauchi et al., 2007). Nakada et al. (2008) reported that hydroxyl radical formed in the liquid droplets might stimulate vegetable

cells, and as a result, the content of AsA was increased in vegetables during storage period after harvest. AsA, as a critical antioxidative component, interacts enzymatically and non-enzymatically with reactive oxygen species (ROS), which is closely related with senescence in plants. The higher AsA content in the electrostatic atomization treated broccoli indicated that electrostatic atomization treatment enhanced the antioxidant capability to scavenge ROS, which contributed to delay the senescence of broccoli. In the meanwhile, AsA is also associated with the biosynthesis of ethylene in plants (Smirnoff and Wheeler, 2000). In the present study, the results showed that in the treatment of electrostatic atomization AsA content was higher than that of control, while the ethylene production was lower than that of the control. This phenomenon suggested that the regulation of AsA metabolism might be closely related with the ethylene production in the electrostatic atomization treatment in broccoli. In addition, although AsA content decreased during the storage period, the content of DHA in broccoli kept almost unchanged at a very low level during the whole storage period, and it was not sensitive to the electrostatic atomization treatment. The similar results also observed in the 1-MCP treated broccoli and cauliflower (Ma et al., 2010; 2011).

AsA metabolism in plants is complex, and its content is controlled by the biosynthesis, oxidation and regeneration processes (Ioannidi et al., 2009; Imai et al., 2009; Li et al., 2010; Ma et al., 2010, 2011). In the recent years, the expression of genes related to AsA metabolism at the transcription level, which might give a precise estimate of antioxidant gene activation, was extensively studied (Nishikawa et al., 2003b; Tokunaga et al., 2005). In the present study, three key genes (*BO-VTC1*,

BO-VTC2 and *BO-GLDH*) in L-galactose pathway, which have been reported to have a major role in regulation AsA biosynthesis, were cloned and their expression was investigated by real-time PCR. The results showed that the expression of *BO-VTC1* and *BO-VTC2* decreased significantly on the first day after harvest. Although the expression of *BO-VTC1* and *BO-VTC2* increased with a peak from the second day, the expression levels of these two genes were much lower than those on the zero day. The expression of *BO-GLDH* decreased rapidly after harvest, and kept at a low level during the storage period in the control. The decreases in the expression of *BO-VTC1*, *BO-VTC2* and *BO-GLDH* were consistent with the decrease of AsA content in broccoli after harvest. With the treatment of electrostatic atomization, the expression of *BO-VTC1*, *BO-VTC2* and *BO-GLDH* was up-regulated. The increases in the gene expression of *BO-VTC1*, *BO-VTC2* and *BO-GLDH* might contribute to the reduction of AsA loss in electrostatic atomization treated broccoli after harvest.

APX is the major enzyme responsible for the AsA oxidation, and comprises a family of isoenzymes distributed in distinct cellular organelles (Panchuk et al., 2002; Ma et al., 2010). Among the APX isoenzymes genes, cytosolic APX has been reported to be responsive to environmental stress, resulting in the relieving the oxidative stress and controlling the AsA content in higher plants (Shigeoka et al., 2002; Jin et al., 2006). In the present study, the results showed that the expression of two cytosolic genes (*BO-APX1* and *BO-APX2*) increased significantly from the second day after harvest, which tended to coincide with the reduction of AsA after harvest. With the treatment of electrostatic atomization, the expression of *BO-APX1* appeared a peak one day earlier

than that of the control, while, the expression of *BO-APX2* and *BO-sAPX* was not affected by the treatment of electrostatic atomization. The regulation of the gene expression of *BO-APX1*, *BO-APX2* and *BO-sAPX* by electrostatic atomization was not well correlated with the higher AsA content in the electrostatic atomization treatment, indicating that the regulation of APX at the transcriptional level might not play an important role in modification of AsA metabolism in response to electrostatic atomization treatment in broccoli after harvest.

MDAR and DHAR are the enzymes responsible for AsA regeneration in plants (Wheeler et al., 1998; Mittler, 2002; Apel and Hirt, 2004; Sairam and Tyagi, 2004). The AsA level in plants can be elevated by increasing the gene expression of the MDAR and DHAR (Chen et al., 2003; Eltayeb et al., 2006). Similar to the three biosynthetic genes (*BO-VTC1*, *BO-VTC2* and *BO-GLDH*), the expression of *BO-MDAR1*, *BO-MDAR2* and *BO-DHAR* decreased after harvest along with the AsA reduction in broccoli after harvest. In the previous study, we found that the regulation of the gene expression of *BO-MDAR1* and *BO-MDAR2* did not closely correlated with modification of AsA content in response to 1-MCP treatment in broccoli and cauliflower (Ma et al., 2010; 2011). However, in the present study the results showed that the regulation of the gene expression of *BO-MDAR1*, *BO-MDAR2* and *BO-DHAR*, which was clearly up-regulated by electrostatic atomization, was in well consistent with the higher AsA content in the electrostatic atomization treated broccoli. These results indicated that the AsA recycling process might play a crucial role in regulation AsA metabolism in response to electrostatic atomization treatment in broccoli after

harvest.

5. Conclusion

In the present study, the results showed that the electrostatic atomization treatment was effective for delaying the senescence of broccoli. With the treatment of electrostatic atomization, the yellowing process was delayed, and the ethylene production and respiration rate were significantly suppressed in broccoli after harvest. In the meanwhile, the electrostatic atomization treatment suppressed the AsA reduction after harvest, which might contribute to the beneficial effects of electrostatic atomization treatment on the senescence of broccoli. Additionally, the modulation of the AsA reduction by electrostatic atomization was highly regulated at the transcription level. The up-regulation of the AsA biosynthetic genes (*BO-VTC1*, *BO-VTC2* and *BO-GLDH*), and AsA regeneration genes (*BO-MDAR1*, *BO-MDAR2* and *BO-DHAR*) led to the suppression of AsA reduction in the electrostatic atomization treated broccoli after harvest. These results indicated that electrostatic atomization treatment might be a new effective approach for delaying the senescence of broccoli. The present study might provide new insights into the application of electrostatic atomization for maintaining the post-harvest quality of fruits and vegetables.

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Table 1 TaqMan MGB probes and primer sequences used for the real-time quantitative RT-PCRs of genes related to ascorbate metabolism.

cDNA (Accession No.)	Primers sequence	TaqMan MGB probe
<i>BO-VTC1</i> (AB716664)	Sense: TTGCAGCAGCTCAAGGACTCT Antisense: TGCCCGATGTCCATCCA	CGCGATGGTGTACC
<i>BO-VTC2</i> (AB716665)	Sense: CAACAGCTTGGGTGCTTTTG Antisense: TGGCCAAGTAATATGCCTGAAA	CACTATCAACCATCTTC
<i>BO-GLDH</i> (Z97060)	Sense: TTGCCCTAGATCCTCTCAATGAC Antisense: TTCCAAAACCTCAGCCTCAGCTT	TCACGTTGGAAAAGTGAA

<i>BO-APX1</i> (AB078599)	Sense: AGCCCATCAGGGAGCAGTT Antisense: CCAGCAAGCTGATGGAAAGCA	CCTACCATCTCCC
<i>BO-APX2</i> (AB078600)	Sense: TGCTCTTAGGTTGTTGGAGCCTAT Antisense: GGAAATCAGCAAAGGAGATGGT	AGAGAGCAGTTCCC
<i>BO-sAPX</i> (AB125635)	Sense: CAAAGAGCTCCTCAACACCAAGT Antisense: ATCATGCCATCCCAAACGA	CCACCCAATTCTGG
<i>BO-MDAR1</i> (AB125636)	Sense: ACGGAATGGCCGATGGT Antisense: GGTCTCTCATAAGGCGCGTAA	TTTGTATTGTCACCAAAGAG
<i>BO-MDAR2</i> (AB125637)	Sense: GAGCACAGAAATAGTGAAAGCAGATC Antisense: TCCCAGCTGCACTGACAA	CGCTGCCAAGACT
<i>BO-DHAR</i> (AB125638)	Sense: TCCATCACCACACCCAACAA Antisense: GCAACACCCTTTGGCAAAA	CTCGGAGACTGCCC

Figure legends

Fig. 1. The main ascorbate metabolism pathway in plants. VTC1, GDP-D-mannose pyrophosphorylase; VTC2, GDP-L-galactose phosphorylase; GLDH, L-galactono-1,4-lactone dehydrogenase; AsA, ascorbic acid; APX, ascorbate peroxidase; MDA, monodehydroascorbate; MDAR, monodehydroascorbate reductase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase.

Fig. 2. Schematic illustration of experimental setup for electrostatic atomization.

Fig. 3. Effect of electrostatic atomization on colour score (A), ethylene production (B) and respiration rate (C) in broccoli. The results shown are the mean \pm SE for triplicate

samples. Means denoted by the same letter did not differ significantly at $P < 0.05$ by Student's t -test. Some error bars and symbols are hidden by symbols.

Fig. 4. Effect of electrostatic atomization on the ascorbate content in broccoli: (A) AsA and (B) DHA. The results shown are the mean \pm SE for triplicate samples. Means denoted by the same letter did not differ significantly at $P < 0.05$ by Student's t -test. Some error bars and symbols are hidden by symbols.

Fig. 5. Effect of electrostatic atomization on the expression of genes related to the AsA metabolism in broccoli. The isoenzymes encoded by discrete genes and distribute in distinct cell organelles. According to the putative localization of the encoding proteins, *BO-VTC1*, *BO-VTC2*, *BO-APX1*, *BO-APX2* and *BO-MDAR2* are cytosolic genes; *BO-GLDH* is mitochondrial gene; and *BO-sAPX*, *BO-MDAR1* and *BO-DHAR* are chloroplastic genes. 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. TaqMan MGB probes and sets of primers used for analysis are shown in Table 1. The results shown are the mean \pm SE for triplicate samples. Means denoted by the same letter did not differ significantly at $P < 0.05$ by Student's t -test. Some error bars and symbols are hidden by symbols.

Fig. 1

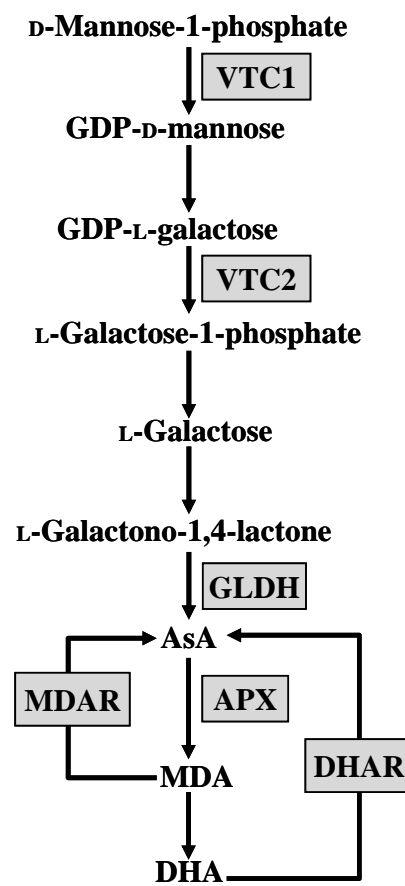


Fig. 2

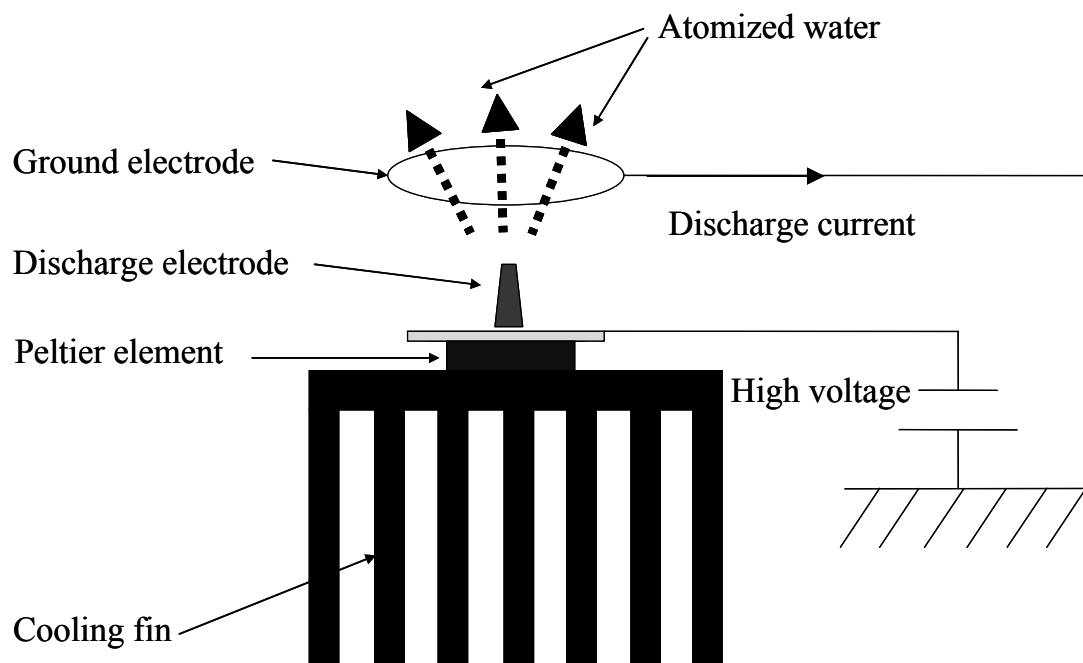


Fig. 3

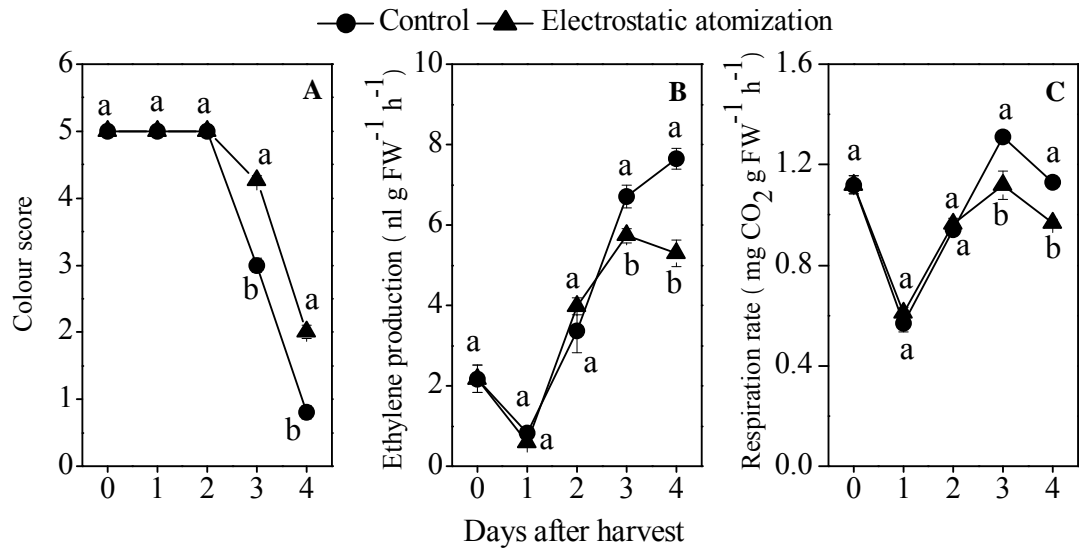
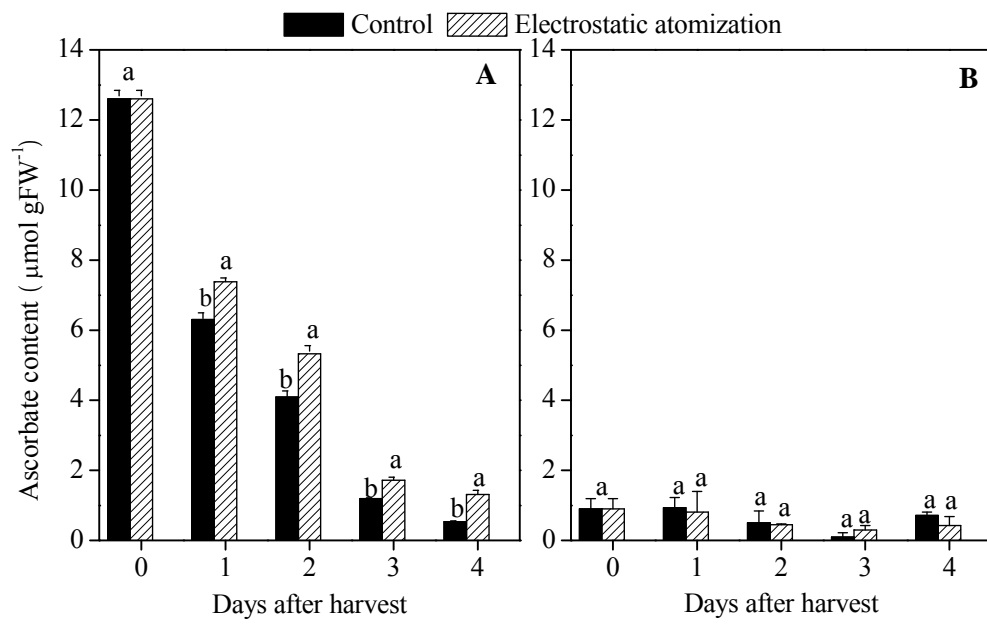
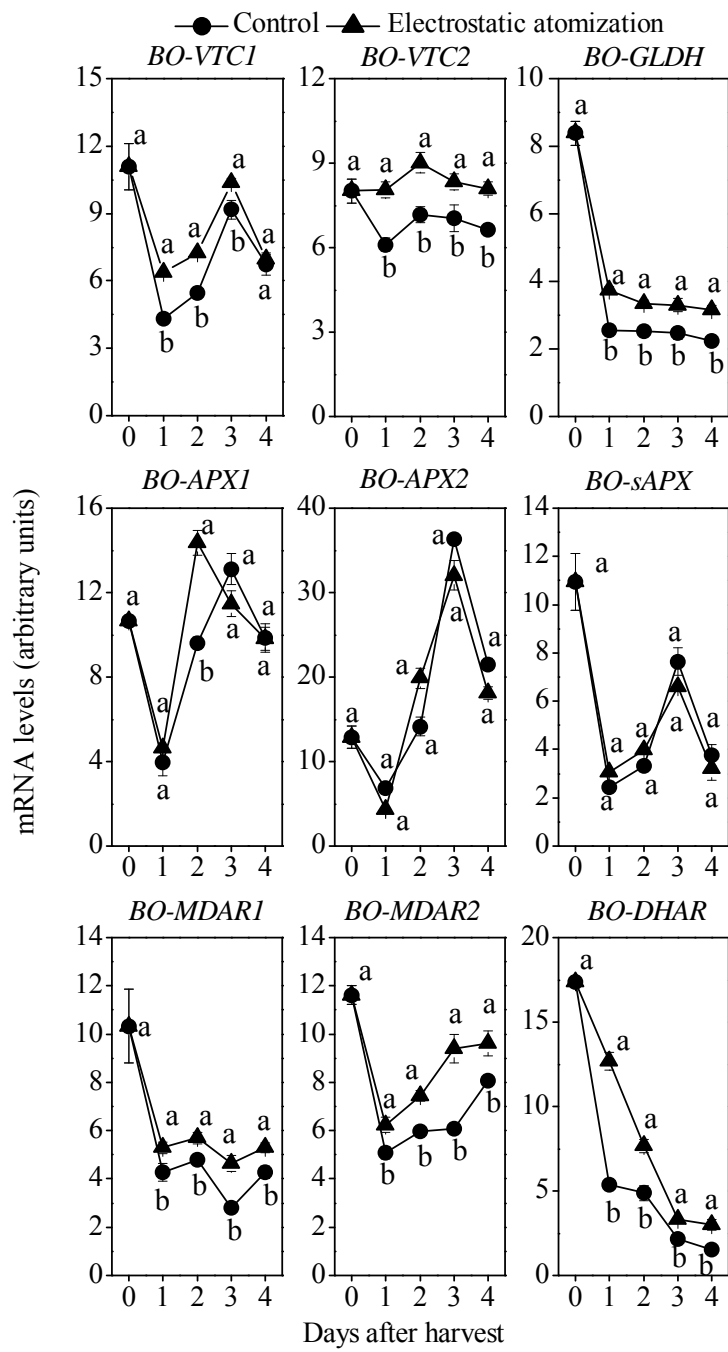


Fig. 4



1 **Fig. 5**



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