

Effect of 1-Methylcyclopropene on the Expression of Genes for Ascorbate Metabolism in Postharvest Cauliflower

Gang Ma^{1,2}, Lancui Zhang², Masaya Kato^{2*}, Kazuki Yamawaki², Tatsuo Asai²,
Fumie Nishikawa³, Yoshinori Ikoma⁴ and Hikaru Matsumoto⁴

¹The United Graduate School of Agricultural Science, Gifu University (Shizuoka University), Yanagido, Gifu 501-1193, Japan

²Faculty of Agriculture, Shizuoka University, Suruga, Shizuoka 422-8529, Japan

³Kuchinotsu Citrus Research Station, National Institute of Fruit Tree Science, Kuchinotsu, Minamishimabara 859-2501, Japan

⁴Okitsu Citrus Research Station, National Institute of Fruit Tree Science, Shimizu, Shizuoka 424-0292, Japan

The effects of 1-methylcyclopropene (1-MCP) on ascorbate (AsA) metabolism were studied and the possible molecular mechanisms were discussed for two cultivars of cauliflower (*Brassica oleracea*, L. var. *botrytis*), ‘Violet Queen’ and ‘Snow Crown’. Ethylene production was suppressed in ‘Violet Queen’, while it was increased in ‘Snow Crown’ by 1-MCP treatment. Meanwhile, the changing patterns of AsA metabolism after harvest were different between the two cauliflower cultivars. In ‘Violet Queen’, AsA content decreased in the control after harvest, and the loss of AsA was delayed by 1-MCP treatment. In ‘Snow Crown’, AsA content remained almost constant and was not affected by 1-MCP treatment. In ‘Violet Queen’, the gene expression of *BO-APX1*, *BO-APX2*, and *BO-sAPX* was down-regulated, while the gene expression of *BO-DHAR* and *BO-GLDH* was up-regulated by 1-MCP treatment. The regulation of these genes contributed to the suppression of AsA reduction in ‘Violet Queen’ treated by 1-MCP. In ‘Snow Crown’, simultaneous down-regulation of *BO-APX1*, *BO-APX2*, and *BO-sAPX*, which were responsible for AsA breakdown, and *BO-MDAR1*, *BO-MDAR2*, *BO-DHAR*, and *BO-GLDH*, which were responsible for AsA regeneration and biosynthesis, might lead to maintain the AsA level constant with 1-MCP treatment.

Key Words: ascorbate, cauliflower, gene expression, 1-methylcyclopropene (1-MCP), senescence.

Introduction

Cauliflower (*Brassica oleracea*, L. var. *botrytis*), a widely consumed vegetable throughout the world, is considered to be a source of nutrition for humans. Epidemiological evidence suggests that consumption of cauliflower can significantly reduce the risks for a number of cancers (Hodges et al., 2006; Lampe and Peterson, 2002). The anticarcinogenic effects of cauliflower are partly attributed to antioxidants, such as glucosinolates, ascorbate acid (AsA), and polyphenols (Carrea and Velasco, 2008; Podsedek, 2007).

AsA is an essential metabolite in plants and plays a crucial role in the antioxidative defense system of plants (Mittler, 2002; Mori et al., 2009). AsA is also known to

act as a cofactor for various enzymes, contribute to the regulation of cell division and expansion, and control the commencement of senescence. In higher plants, AsA metabolism is complex, and is controlled by the processes of biosynthesis, catabolism, and regeneration. L-Galactono-1,4-lactone dehydrogenase (GLDH), a key enzyme on the inner mitochondrial membrane, catalyzes the oxidation of the last precursor L-galactono-1,4-lactone to AsA. Enzyme activity and the transcription level of GLDH have been reported to positively correlate with the AsA level in tobacco and *Arabidopsis thaliana* (Gatzek et al., 2002; Kato and Esaka, 1999; Tabata et al., 2001). Additionally, the ascorbate-glutathione cycle, which is the oxidation and recycling pathway of AsA, also plays an important role in regulating AsA metabolism in plants. In this pathway, the enzymatic action of ascorbate peroxidase (APX) produces monodehydroascorbate (MDA), which can dismutate spontaneously to AsA and dehydroascorbate (DHA) or be reduced enzymatically to AsA by NADPH-dependent

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* Corresponding author (E-mail: amkato@ipc.shizuoka.ac.jp).

monodehydroascorbate reductase (MDAR). DHA is also reduced to AsA enzymatically in a reaction mediated by dehydroascorbate reductase (DHAR) (Ma et al., 2010; Nishikawa et al., 2003a; Noctor and Foyer, 1998). The enzymes in the ascorbate-glutathione cycle exist as isoenzymes, which are distributed in distinct cellular organelles: chloroplasts, plastids, mitochondria, and peroxisomes (Mittova et al., 2000). In the previous study, the genes of the isoenzymes involved in the ascorbate-glutathione cycle have been isolated and well characterized in broccoli after harvest. The regulation of gene expression appeared to be an important mechanism by which AsA metabolism was regulated during the senescence process in broccoli (Ma et al., 2010; Nishikawa et al., 2003a).

Generally, AsA content decreases gradually in fruits and vegetables after harvest. The loss of AsA could be reduced through postharvest treatments, such as modification of the atmosphere and low temperature treatment (Mori et al., 2009; Shigenaga et al., 2005). 1-Methylcyclopropene (1-MCP), which is an effective inhibitor of ethylene action and binds irreversibly to ethylene receptors, has been shown to be potentially useful for delaying ripening, maintaining quality, and extending the shelf-life of fruits, vegetables, and ornamental crops (Tatsuki, 2010; Watkins, 2008). Application of 1-MCP can delay yellowing, decrease respiration and decay, alleviate certain ethylene-induced postharvest physiological disorders, and extend the shelf-life of broccoli (Able et al., 2002; Fan and Mattheis, 2000; Gong and Mattheis, 2003; Ku and Wills, 1999; Ma et al., 2009). In our previous study, we found that 1-MCP delayed the loss of AsA in broccoli after harvest, and the modulation of AsA metabolism contributed to the beneficial effects of 1-MCP on senescence (Ma et al., 2010). Similar to broccoli, cauliflower is a highly perishable horticultural crop with a short shelf-life, however, as far as we know, there is limited information about the application of 1-MCP to cauliflower. In the present study, the effects of 1-MCP on ethylene production and AsA content were investigated for two cauliflower cultivars ‘Violet Queen’, which is purple, and ‘Snow Crown’, which is white to cream. Moreover, to further investigate the regulatory mechanism of AsA metabolism by 1-MCP treatment, the expression of genes (*BO-APX1*, *BO-APX2*, *BO-sAPX*, *BO-MDAR1*, *BO-MDAR2*, *BO-DHAR*, and *BO-GLDH*) directly related to AsA metabolism was analyzed by real-time PCR in the two cauliflower cultivars.

Materials and Methods

Plant materials and treatments

Two cultivars of cauliflower (*Brassica oleracea*, L. var. *botrytis*), ‘Violet Queen’ and ‘Snow Crown’, were grown at Fujieda Farm of Shizuoka University, Shizuoka, Japan under the same field conditions. Six harvested cauliflower heads, which were at the same stage of

maturation, were placed in 50-L sealed plastic chambers. The cauliflower heads were continuously treated as follows: in air as a control; with $5\ \mu\text{L}\cdot\text{L}^{-1}$ of 1-MCP (Rohm and Hass, Japan). 1-MCP treatment was performed twice a day to exchange the air in the chambers. The air was also exchanged by opening the chambers twice a day in the control. In each chamber, 10% KOH solution was placed to absorb CO_2 . Three cauliflower heads were used each time. All treatments were conducted in the chambers at 20°C under humidified conditions ($\text{RH} > 95\%$). Florets excised from the heads with a single-edged razor were immediately frozen in liquid nitrogen, except for the sample for ethylene production analyses, and stored at -80°C until used.

Measurements of ethylene production

One gram of florets was placed in a 15-mL vial immediately after excising from the cauliflower head. The vial was sealed using a silicon rubber cap, and the 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, Hitachi, Japan) equipped with an alumina column at 70°C and a flame ionization detector, in accordance with the procedures described by Nishikawa et al. (2001). The rate of ethylene production was expressed as nmol ethylene per h per g FW.

Extraction and assays of ascorbate

The ascorbate content of the reduced and oxidized forms was assayed by HPLC in accordance with the method described by Nishikawa et al. (2001). Each frozen sample was homogenized using a mortar and pestle in 10 volumes of 2% metaphosphoric acid. The homogenate was centrifuged at $14,000 \times g$ for 20 min, and then the supernatant was filtered through Miracloth (Calbiochem, Germany). The pH of the filtrate was adjusted by adding an equal volume of 0.2 M K-phosphate buffer (pH 7.5). Total ascorbate was assayed by adding 1 mL of 2 mM dithiothreitol (DTT) to an aliquot of filtrate and incubating the mixture for 15 min (Masuda et al., 1988). After the sample was filtered through a $0.2\text{-}\mu\text{m}$ cellulose acetate filter (Advantec, Japan), a $20\ \mu\text{L}$ aliquot was injected onto a TSK-GEL (Amide-80) column (Tosoh, Japan) attached to a LC-10AD pump (Shimadzu, Japan). The column kept at 20°C was eluted with 80% acetonitrile: 0.04% phosphoric acid at a flow rate of $1.0\ \text{mL}\cdot\text{min}^{-1}$. Ascorbate was monitored at 245 nm (retention time 5.3 min) 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 ascorbate to construct a standard curve. AsA content was determined in a similar manner without the addition of DTT. DHA content was calculated by subtracting the AsA value from the total ascorbate.

Isolation and sequence analysis of genes related to ascorbate metabolism

Total RNA was extracted from cauliflower florets in accordance with the method described by Kato et al. (2000). First-strand cDNA was synthesized from 2 µg total RNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, USA).

For each cultivar, the cDNA fragments of genes related to ascorbate metabolism were amplified by PCR. The primers were the same as those used in broccoli (Ma et al., 2010). The nucleotide sequences of the cDNA fragments of genes related to ascorbate metabolism in cauliflower ('Violet Queen' and 'Snow Crown') showed high identity (>98.0% at nucleotide sequence level) to those of corresponding cDNAs in broccoli. The amplified cDNAs were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). TaqMan MGB probes and sets of primers for *BO-APX1*, *BO-APX2*, *BO-sAPX*, *BO-MDAR1*, *BO-MDAR2*, *BO-DHAR*, and *BO-GLDH* were designed on the basis of the common sequences using Primer Express software (Applied Biosystems). The sequences of TaqMan MGB probes and sets of primers were the same as those of broccoli used in a previous study (Ma et al., 2010). According to the putative localization of the encoding proteins, *BO-APX1*, *BO-APX2*, and *BO-MDAR2* are cytosolic genes; *BO-GLDH* is a mitochondrial gene; and *BO-sAPX*, *BO-MDAR1*, and *BO-DHAR* are chloroplastic genes.

Total RNA extraction and real-time quantitative RT-PCR

Total RNA was extracted from the cauliflower florets after harvest in accordance with the method described by Kato et al. (2000). The total RNA was cleaned up using the RNeasy Mini Kit (Qiagen, Germany) with DNase digestion on a column in accordance with the manufacturer's instructions. Reverse transcription was performed with 2 µg purified RNA and a random hexamer in 60 min at 37°C using TaqMan Reverse Transcription Reagents (Applied Biosystems).

TaqMan real-time PCR was carried out with a TaqMan MGB probe, a set of primers, and TaqMan Universal PCR Master Mix (Applied Biosystems) in accordance with the manufacturer's instructions. As an endogenous control, a TaqMan Ribosomal RNA Control Reagents VIC probe (Applied Biosystems) was used. 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. Gene expression was analyzed with ABI PRISM 7300 Sequence Detection System Software (Applied Biosystems) and normalized with the results for 18S ribosomal RNA. The gene expression level was expressed relative to the level of 18S ribosomal RNA using arbitrary units. Real-time quantitative RT-PCR was performed with three replicates for each sample.

Statistical analysis

All values are shown as the mean ± SE for three replicates. The data were analyzed, and Student's *t*-test was used to compare the treatment means at $P < 0.05$ and $P < 0.01$.

Results

Effect of 1-MCP treatment on visual quality

In the present study, noticeable color changes were not observed in the florets of 'Violet Queen' and 'Snow Crown' during the experimental period both in the control and with 1-MCP treatment (Fig. 1). In the florets of 'Violet Queen', the color remained purple during the experimental period. In the florets of 'Snow Crown', the color changed slightly from white to pale yellow after harvest.

Effect of 1-MCP treatment on ethylene production

In 'Violet Queen', ethylene production increased rapidly, reaching a peak on the third day after harvest. Compared with the control, ethylene production was significantly suppressed by 1-MCP treatment on the third day, and no clear peaks were observed during the experimental period in 'Violet Queen' (Fig. 2A). In 'Snow Crown', ethylene production was lower than in 'Violet Queen', and no noticeable peak of ethylene production was observed throughout the experimental period. With 1-MCP treatment, ethylene production increased after harvest with a peak on the first day (Fig. 2B).

Effect of 1-MCP treatment on AsA content

In 'Violet Queen', AsA content decreased gradually during the experimental period. Treatment with 1-MCP clearly slowed the AsA reduction after harvest (Fig. 3A). In 'Snow Crown', AsA content, which was lower than in 'Violet Queen', remained almost constant throughout the experimental period. Compared with the control, AsA content was not significantly affected by 1-MCP treatment in 'Snow Crown' (Fig. 3B).

The content of DHA was relatively low in the two cultivars investigated in the present study, and all exhibited less than 10% total ascorbate throughout the postharvest period. The content of DHA remained almost unchanged in the control and 1-MCP-treated cauliflower florets throughout the experimental period (Fig. 3).

Effect of 1-MCP on expression of genes related to AsA metabolism

In the control of 'Violet Queen', the expression of the two cytosolic genes, *BO-APX1* and *BO-APX2*, decreased slightly after harvest, and then increased significantly with a peak on the third and second days, respectively (Fig. 4). The gene expression of *BO-sAPX*, which is the stromal APX in chloroplasts, decreased rapidly after harvest, and then increased slightly. The gene expression levels of *BO-APX1* on the third day, *BO-APX2* on the

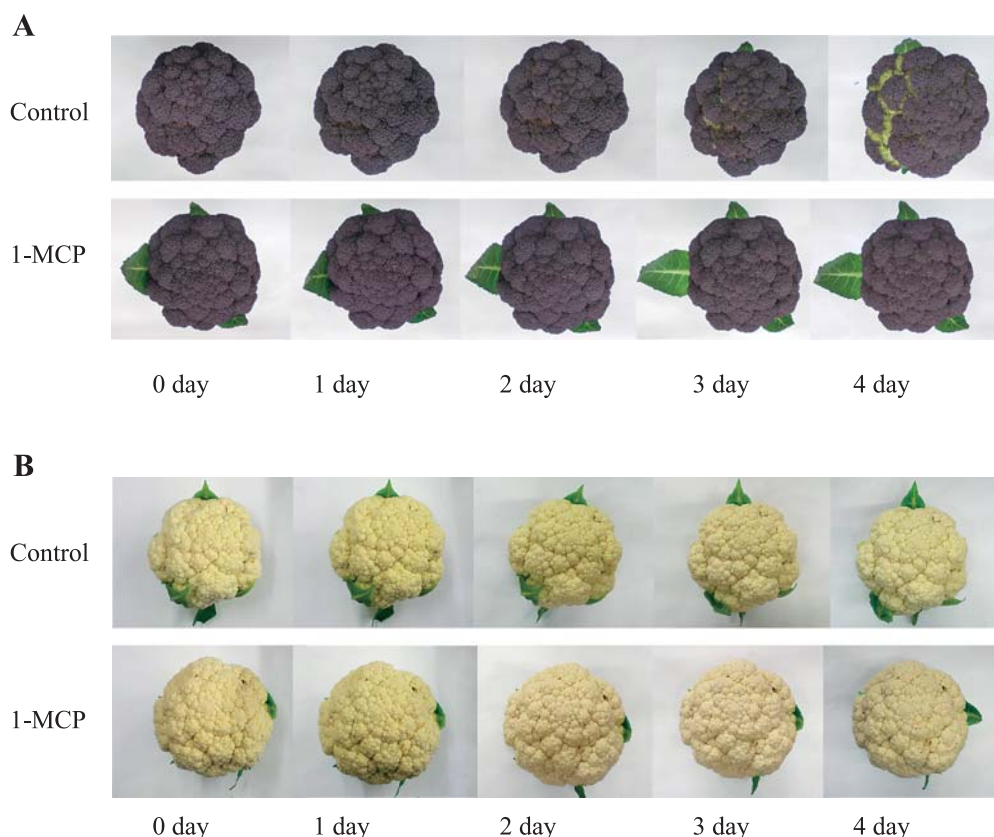


Fig. 1. Effect of 1-MCP treatment on visual quality in cauliflower: (A) 'Violet Queen' and (B) 'Snow Crown'.

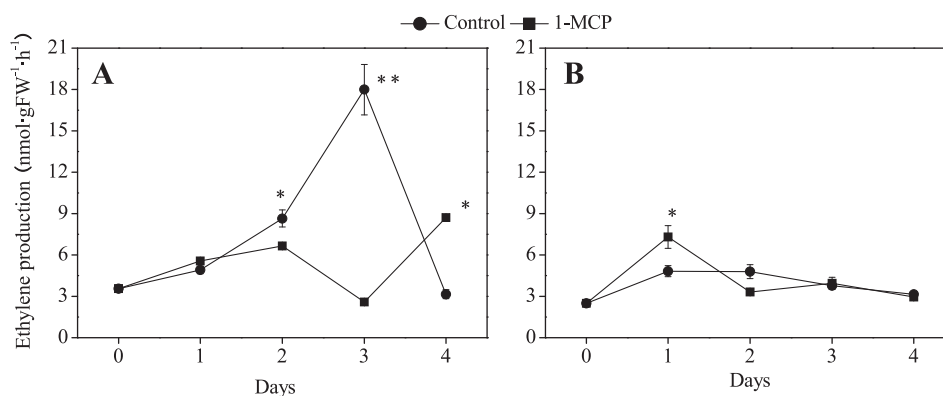


Fig. 2. Effect of 1-MCP on ethylene production in cauliflower: (A) 'Violet Queen' and (B) 'Snow Crown'. The results are the mean \pm SE for triplicate samples. * and ** indicate significances between control and 1-MCP treatment on the same day at $P < 0.05$ and $P < 0.01$ by Student's *t*-test, respectively. Some error bars and symbols are hidden by symbols.

second day and *BO-sAPX* from the second to fourth day of 1-MCP treatment were lower than those in the control after harvest (Fig. 4). In 'Snow Crown', the gene expression of *BO-APX1* and *BO-APX2* decreased after harvest, and then increased, with a peak on the second day (Fig. 5). Similar to 'Violet Queen', the gene expression of *BO-sAPX* decreased rapidly after harvest, and then increased slightly in 'Snow Crown'. With 1-MCP treatment, the gene expression levels of *BO-APX1* and *BO-APX2* were lower than those of the control on the second and third days, while the gene expression of

BO-sAPX was not clearly affected by 1-MCP treatment compared with the control during the experimental period (Fig. 5).

The gene expression of *BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR*, which were related to AsA regeneration, decreased rapidly after harvest in the control of 'Violet Queen' (Fig. 4). With 1-MCP treatment, the gene expression level of *BO-MDAR2* was lower than that of the control on the second and fourth days, while the gene expression of *BO-MDAR1* was not clearly affected by 1-MCP treatment throughout the experimental period

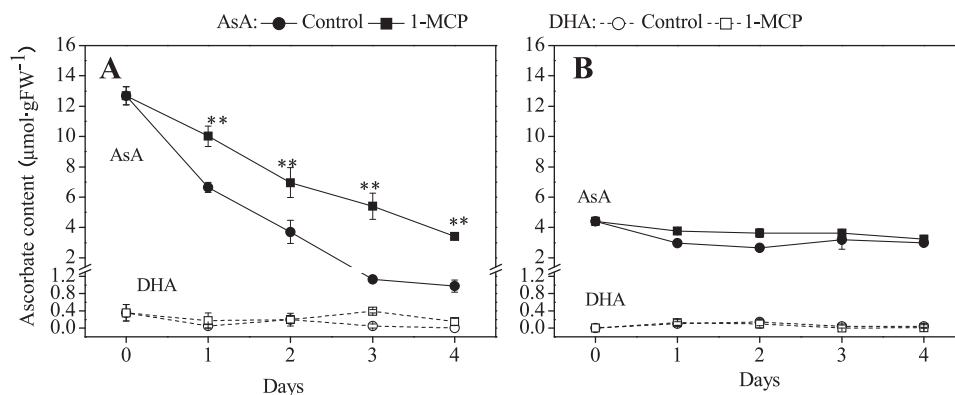


Fig. 3. Effect of 1-MCP on ascorbate content in cauliflower: (A) 'Violet Queen' and (B) 'Snow Crown'. The results are the mean \pm SE for triplicate samples. * and ** indicate significances between control and 1-MCP treatment on the same day at $P < 0.05$ and $P < 0.01$ by Student's t -test, respectively. Some error bars and symbols are hidden by symbols.

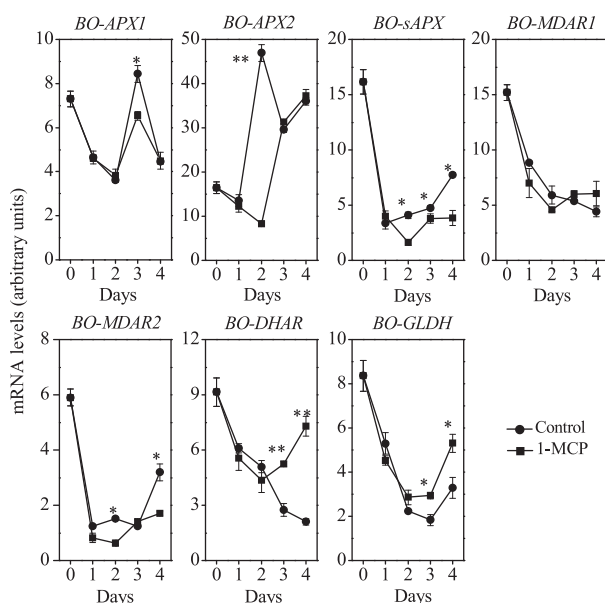


Fig. 4. Effect of 1-MCP on the expression of genes related to AsA metabolism in 'Violet Queen'. The isoenzymes are encoded by discrete genes and are distributed in distinct cell organelles. According to the putative localization of the encoding proteins, *BO-APX1*, *BO-APX2*, and *BO-MDAR2* are cytosolic genes; *BO-GLDH* is a 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. The gene expression level was expressed relative to the level of 18S ribosomal RNA using arbitrary units. The results are the mean \pm SE for triplicate samples. * and ** indicate significances between control and 1-MCP treatment on the same day at $P < 0.05$ and $P < 0.01$ by Student's t -test, respectively. Some error bars and symbols are hidden by symbols.

in 'Violet Queen'. Compared with the control, the gene expression level of *BO-DHAR* in the treatment of 1-MCP was higher on the third and fourth days in 'Violet Queen' (Fig. 4). In the control of 'Snow Crown', the gene expression of *BO-MDAR1* and *BO-MDAR2* increased, with a peak on the first day after harvest. The

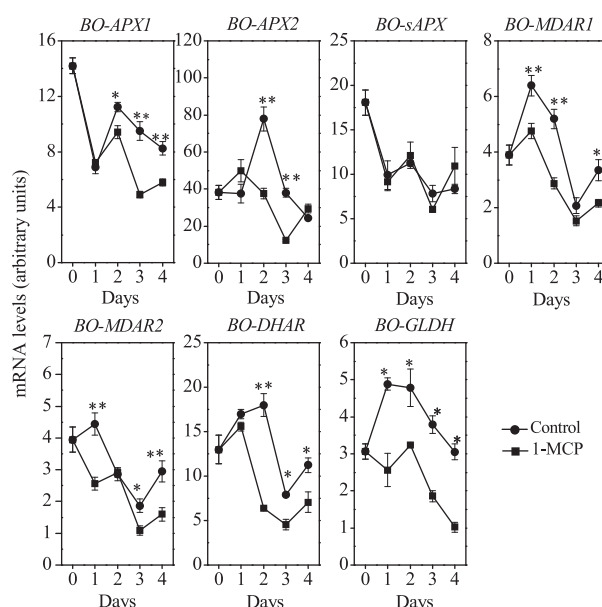


Fig. 5. Effect of 1-MCP on the expression of genes related to AsA metabolism in 'Snow Crown'. The isoenzymes are encoded by discrete genes and are distributed in distinct cell organelles. According to the putative localization of the encoding proteins, *BO-APX1*, *BO-APX2*, and *BO-MDAR2* are cytosolic genes; *BO-GLDH* is a 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. The gene expression level was expressed relative to the level of 18S ribosomal RNA using arbitrary units. The results are the mean \pm SE for triplicate samples. * and ** indicate significances between control and 1-MCP treatment on the same day at $P < 0.05$ and $P < 0.01$ by Student's t -test, respectively. Some error bars and symbols are hidden by symbols.

gene expression of *BO-DHAR* increased clearly after harvest, reaching a peak on the second day. With 1-MCP treatment, the gene expression levels of *BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR* were much lower than those of the control in 'Snow Crown' (Fig. 5).

In the control of 'Violet Queen', the gene expression

of the mitochondrial gene (*BO-GLDH*) decreased clearly after harvest, and reached its lowest level on the third day. With 1-MCP treatment, the gene expression level of *BO-GLDH* was higher than that of the control from the second to fourth day in ‘Violet Queen’ (Fig. 4). In contrast to ‘Violet Queen’, in the control of ‘Snow Crown’, the gene expression of *BO-GLDH* increased rapidly with a peak on the first day after harvest. The gene expression level of *BO-GLDH* with 1-MCP treatment was lower than that of the control throughout the experimental period (Fig. 5).

Discussion

Changes in the ethylene production

1-MCP, which acts as an efficient ethylene antagonist, has been applied to maintain the quality of fruits and vegetables by inhibiting ethylene production and alleviating certain ethylene-induced postharvest physiological disorders during storage. By 2007, 1-MCP had been registered for use on more than 50 fruits and vegetables (Watkins, 2008); however, so far, the application of 1-MCP to cauliflower after harvest has not been reported. Endogenous ethylene is closely associated with the senescence of vegetables after harvest (Hyodo et al., 1994; Kasai et al., 1996; King and Morris, 1994). Suppression of ethylene production by 1-MCP led to the inhibition of some metabolic activity and the senescence of broccoli (Ma et al., 2009, 2010). In the present study, the results showed that two cauliflower cultivars, ‘Violet Queen’ and ‘Snow Crown’, exhibited different changes in the pattern of ethylene production after harvest (Fig. 2). In ‘Violet Queen’, ethylene production increased rapidly, reaching a peak on the third day after harvest. With 1-MCP treatment, ethylene production was suppressed and no clear peaks were observed during the experimental period, which indicated that the application of 1-MCP was effective for delaying senescence in ‘Violet Queen’ (Fig. 2A). In contrast to ‘Violet Queen’, in ‘Snow Crown’, which did not show an ethylene climacteric peak during storage, ethylene production was promoted by 1-MCP treatment (Fig. 2B). Similar responses to 1-MCP treatment have been reported in the calyx of detached young persimmon fruit (Nakano et al., 2003) and *Pelargonium* cuttings (Mutui et al., 2007). It has been suggested that regulation of the ethylene auto-inhibition effect, which was relieved by 1-MCP, might result in the observed increases in ethylene production.

Changes in the AsA content

Fruits and vegetables are a rich source of nutrition for humans, supplying minerals, vitamins, and chemopreventive agents, such as carotenoids and polyphenols. In recent years, in addition to investigations to improve the organoleptic qualities of plants, extensive efforts have been devoted to improving the nutrition contents of fruits and vegetables (Giovannoni, 2001; Ioannidi et al., 2009;

Lee and Kader, 2000; Lo Scalzo et al., 2008; Ohkawa et al., 2009; Sugiyama et al., 2010; Yaguchi et al., 2009). AsA is one of the most important nutrients in plants and has many biological activities beneficial to humans and animals; however, AsA is sensitive to various factors, such as preharvest climatic conditions, harvesting methods, and postharvest treatments (Mori et al., 2009; Shigenaga et al., 2005). In broccoli florets, the AsA content declined to a low level during storage after harvest (Nishikawa et al., 2003a), while loss of AsA could be reduced by postharvest handling, such as heat and ethanol vapor treatments (Mori et al., 2009; Shigenaga et al., 2005). In our previous study, we found that AsA reduction was clearly slowed by 1-MCP treatment in two broccoli cultivars, ‘Haitsu’ and ‘Ryokurei’. Moreover, the regulation of AsA content, which is a key antioxidant in plants, might partly contribute to the modulation of senescence by 1-MCP treatment in ‘Haitsu’ and ‘Ryokurei’ (Ma et al., 2010). In the present study, we found that the changes in AsA metabolism varied between the two cauliflower cultivars, ‘Violet Queen’ and ‘Snow Crown’, after harvest. In ‘Violet Queen’, AsA content decreased rapidly after harvest and the loss of AsA was suppressed by 1-MCP treatment (Fig. 3A). The changes in AsA content in untreated and 1-MCP-treated ‘Violet Queen’ were similar to those in broccoli (Ma et al., 2010). The suppression of AsA loss after harvest by 1-MCP might contribute to delaying senescence in ‘Violet Queen’. In ‘Snow Crown’, which accumulated lower AsA, the content of AsA remained almost constant in the control and 1-MCP-treated samples (Fig. 3B). In addition, we found that the content of DHA remained almost unchanged at a very low level in the control and with 1-MCP treatment in the two cauliflower cultivars. It has been reported that the instability of DHA and different metabolites, such as oxalic and L-threonic acids, produced by AsA breakdown by means of enzymatic cleavage of particular carbon-carbon bonds might contribute to the reduction of AsA without an increase of DHA (De Pinto et al., 2000; Ma et al., 2010; Saito et al., 1997).

Changes in the expression of genes related to AsA metabolism

The enzymes involved in the ascorbate-glutathione cycle have been reported, although relatively little information is available on other enzymes related to AsA biosynthesis and DHA decomposition (Noctor and Foyer, 1998). The enzymes in the ascorbate-glutathione cycle, such as APX, MDAR, DHAR, and glutathione reductase, exist as isoenzymes encoded by discrete genes and are distributed in distinct cellular compartments, and it is difficult to assay the activity of each isoenzyme separately. In recent years, the gene expression of each isoenzyme, which might give a more precise estimate of antioxidant gene activation, has been studied

extensively (Nishikawa et al., 2003b; Tokunaga et al., 2005). In plants, APX is the key enzyme in the H_2O_2 -detoxification system in cytosol, chloroplasts, mitochondria, and peroxisome, constituting an important component of the ascorbate-glutathione cycle. It has been reported that different APX isogenes behaved in a different way at the transcription level in *Arabidopsis thaliana* and broccoli (Ma et al., 2010; Panchuk et al., 2002). In the present study, we found that the gene expression of *BO-APX1* and *BO-APX2* decreased slightly after harvest, and then increased with a peak during the storage period in the two cauliflower cultivars, 'Violet Queen' and 'Snow Crown' (Figs. 4 and 5). In contrast to cytosolic APX, the expression of the chloroplastic gene *BO-sAPX* decreased sharply after harvest, and then increased slightly in the two cauliflower cultivars. In 'Violet Queen', the gene expression of *BO-APX1* and *BO-APX2* was down-regulated by 1-MCP on the third and second day, respectively. The gene expression of *BO-sAPX* was down-regulated from the second day. Thus, down-regulation of the expression of *BO-APX1*, *BO-APX2*, and *BO-sAPX* might contribute to the higher AsA level in 'Violet Queen' treated by 1-MCP. In 'Snow Crown', with 1-MCP treatment, the gene expression of *BO-APX1* and *BO-APX2* was down-regulated, while the gene expression of *BO-sAPX* was not affected markedly. The different regulations of APX in cytosol and chloroplasts in response to 1-MCP treatment were also observed in broccoli (Ma et al., 2010).

It has been confirmed that the expression of genes encoding enzymes associated with AsA regeneration, MDAR and DHAR, and of the gene encoding the enzyme associated with AsA biosynthesis, GLDH, is closely related to the changes in AsA content in higher plants (Apel and Hirt, 2004; Chen et al., 2003; Mittler, 2002; Sairam and Tyagi, 2004; Wheeler et al., 1998). Nishikawa et al. (2003a) reported that the down-regulation of *BO-MDAR* and *BO-DHAR* directly led to ascorbate degradation in broccoli after harvest. In melon, up-regulation of the gene expression of GLDH coincided with elevated levels of AsA (Pateraki et al., 2004). In our previous study, we found that regulation of the gene expression of *BO-GLDH* was closely related to AsA content in the control and 1-MCP-treated broccoli (Ma et al., 2010). In the present study, the results showed that in the control of 'Violet Queen', the expression of the three genes encoding enzymes associated with AsA regeneration (*BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR*) and of the gene encoding the enzyme associated with AsA biosynthesis (*BO-GLDH*) decreased noticeably after harvest, which coincided with the loss of AsA after harvest. In 'Violet Queen', with 1-MCP treatment, the gene expression of *BO-MDAR1* was not affected, while the gene expression of *BO-MDAR2* was slightly down-regulated. The regulation of the gene expression of *BO-MDAR1* and *BO-MDAR2* by 1-MCP was not well correlated with higher AsA content with 1-MCP

treatment, indicating that the regulation of MDAR at the transcriptional level might not play an important role in the modification of AsA metabolism in response to 1-MCP in 'Violet Queen'. Similar results were observed in the two broccoli cultivars, 'Haitsu' and 'Ryokurei' (Ma et al., 2010). The gene expression of *BO-DHAR* and *BO-GLDH*, which was up-regulated by 1-MCP treatment, was closely related to the higher AsA content in 1-MCP-treated 'Violet Queen' in the later stage of the experimental period. On the first day, the reduction of the AsA level was delayed by 1-MCP treatment, although the levels of the gene expression of *BO-DHAR* and *BO-GLDH* were similar between 1-MCP treatment and the control. It is possible that other genes involved in AsA metabolism, such as the VTC gene family (upstream regulating genes of GLDH) which is in the L-galactose pathway responsible for AsA biosynthesis, might be related to changes in AsA content in the initial stages. In contrast to 'Violet Queen', in the control of 'Snow Crown', the gene expression of three AsA regeneration genes (*BO-MDAR1*, *BO-MDAR2*, and *BO-DHAR*) and the AsA biosynthesis gene *BO-GLDH* increased and reached a peak after harvest. The increased expression of these genes contributed to maintain the AsA content constant after harvest in 'Snow Crown'. With 1-MCP treatment, the gene expression levels of *BO-MDAR1*, *BO-MDAR2*, *BO-DHAR*, and *BO-GLDH* were lower than those of the control. Interestingly, we found that in 'Snow Crown' the expression of genes investigated in the present study was down-regulated by 1-MCP treatment, except for *BO-sAPX*. Thus, the simultaneous down-regulation of AsA breakdown genes (*BO-APX1* and *BO-APX2*), and AsA regeneration and biosynthesis genes (*BO-MDAR1*, *BO-MDAR2*, *BO-DHAR*, and *BO-GLDH*), contributed to keep the AsA content constant in the 1-MCP-treated 'Snow Crown'. Further research is needed to explain this phenomenon by investigating the enzyme activities associated with AsA breakdown, regeneration and biosynthesis.

In conclusion, we investigated the effects of 1-MCP on AsA metabolism in two cauliflower cultivars, 'Violet Queen' and 'Snow Crown'. The results showed that AsA metabolism was different in the two cauliflower cultivars. In 'Violet Queen', AsA content decreased in the control after harvest, and the loss of AsA was delayed by 1-MCP treatment, while in 'Snow Crown', the AsA content remained constantly low and was less sensitive to 1-MCP treatment. Additionally, the results of the gene expression suggested that the modification of AsA content was highly regulated at the transcription level in the two cauliflower cultivars. In 'Violet Queen', the down-regulation of *BO-APX1*, *BO-APX2*, and *BO-sAPX*, and up-regulation of *BO-DHAR* and *BO-GLDH* led to higher AsA content with 1-MCP treatment. In 'Snow Crown', simultaneous down-regulation of *BO-APX1*, *BO-APX2*, and *BO-sAPX*, which were responsible for AsA breakdown, and *BO-MDAR1*, *BO-MDAR2*, *BO-*

DHAR, and *BO-GLDH*, which were responsible for AsA regeneration and biosynthesis, contributed to maintain the AsA level constant with 1-MCP treatment. These results might provide new insights into the application of 1-MCP to maintain the AsA level in horticultural crops after harvest.

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