Methyl Salicylate-Induced Arginine Catabolism Is Associated with Up-regulation of Polyamine and Nitric Oxide Levels and Improves Chilling Tolerance in Cherry Tomato Fruit

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ABSTRACT: The effects of methyl salicylate (MeSA) on chilling injury (CI) and gene expression levels, enzyme activities, and metabolites related to arginine catabolism in cherry tomato fruit were investigated. Freshly harvested fruits were treated with 0.05 mM MeSA vapor at 20 °C for 12 h and then stored at 2 °C for up to 28 days. MeSA reduced CI and enhanced the accumulation of putrescine, spermidine, and spermine, which was associated with increased gene expression levels and activities of arginase, arginine decarboxylase, and ornithine decarboxylase at most sampling times. MeSA also increased nitric oxide synthase activity, which at least partly contributed to the increased nitric oxide content. The results indicate that MeSA activates the different pathways of arginine catabolism in cold-stored fruit and that the reduction in CI by MeSA may be due to the coordinated metabolism of arginine and the increase in polyamines and nitric oxide levels.

KEYWORDS: methyl salicylate, arginine catabolism, cherry tomato fruit, chilling injury, polyamines, nitric oxide

INTRODUCTION

There is widespread interest in L-arginine because it is involved in multiple metabolic processes in living cells. In addition to serving as a component of proteins, arginine is a precursor for the biosynthesis of polyamines, agmatine, and proline, as well as the cell-signaling molecules glutamate, γ-aminobutyric acid, and nitric oxide (NO).[1−3] Arginase, arginine decarboxylase (ADC), and nitric oxide synthase (NOS) are the key enzymes in arginine catabolism (Figure 1).[1] ADC catalyzes arginine to produce polyamines, whereas arginase hydrolyzes arginine to urea and ornithine, the latter of which is a precursor for polyamine biosynthesis via ornithine decarboxylase (ODC) (Figure 1).[1] In mammalian cells, most of the NO produced is due to NOS, which catalyzes arginine to produce NO and citrulline.[4] Although the genes encoding NOS proteins and animal-type NOS in higher plants remain elusive,[5,6] an arginine-dependent NO synthesis, similar to that in animals, has also recently been described in plants.[7−9] In animal systems, the metabolism of arginine has been a field of active research for many years, and a plethora of data has been gathered in the basic and clinical sciences.[1,4] However, in contrast to current information on arginine metabolism in animals, very little is known about this topic in plants, especially its possible roles in plant stress responses.

Chilling injury (CI) is a major postharvest problem for tropical and subtropical horticultural products subjected to low-temperature storage. Salicylic acid (SA) is regarded as an endogenous plant hormone that plays an important role in regulating stress response and plant growth and development.[10,11] Recently, a few studies have reported that treatment with SA and methyl salicylate (MeSA) can reduce the development of CI symptoms in a number of horticultural crops, including loquat, tomato, pomegranate, and peach fruit.[12−15] However, the mode of action of MeSA in reducing CI and quality deterioration has not been clearly elucidated. NO and polyamines are both important signaling molecules implicated in numerous physiological and biological processes during plant development and stress response.[16,17] A role for endogenous or exogenous polyamines or NO in increasing horticultural crops tolerance to cold stress has been reported.[17−20] It is known that arginine is the primary precursor for biosynthesis of polyamines and NO. Moreover, treatment with exogenous arginine can alleviate the CI of cherry tomato fruit during cold storage.[21] Consequently, arginine metabolism appears to be an interesting metabolic pathway potentially involved in the chilling tolerance of plant. However, little information is available about the effects MeSA treatments as a factor that affects arginine catabolism in fruit during cold storage.

Cherry tomato fruit, a typical chilling-sensitive horticulture crop, is an excellent model system to investigate the physiological and molecular mechanism of postharvest CI in fruit during cold storage. The present work was undertaken to evaluate the effect of MeSA treatment on CI, gene expression, and activities of enzymes involved in arginine catabolism as well as the polyamines and NO levels in cherry tomato fruit during storage at 2 °C. Our objectives were to investigate, first, whether the plant regulator is involved in coordinating induction of different pathways of arginine catabolism and, second, whether the arginine catabolism might be involved in MeSA-induced chilling tolerance mechanisms.

MATERIALS AND METHODS

Fruits and MeSA Treatments. Cherry tomato (*Solanum lycopersicum* L. cv. Messina) fruits were harvested at the mature green stage.
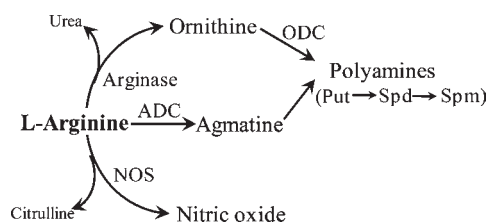
Figure 1. Arginine catabolism and the coordinate biosynthesis of polyamines and nitric oxide in plants. For the sake of clarity, not all reactants and products are shown. ADC, arginine decarboxylase; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; Put, putrescine; Spd, spermidine; Spm, spermine.

Blemish-free fruits of the same size and shape were selected and randomly divided into two lots of 180 fruit each. Fruits were placed in 50 L airtight containers. MeSA was spotted onto filter paper at final vapor concentrations of 0.0 (control) or 0.05 mM and incubated for 12 h in darkness at 20 °C. Afterward, the containers were opened, and the fruits were stored at 2 ± 1 °C with a relative humidity of 80–90% for up to 28 days. Three replicates were conducted in this experiment.

Untreated and treated fruits were sampled immediately following treatment or after 1, 3, 7, 14, 21, and 28 days of cold storage. Equatorial slices of 10 fruit per replicate were diced, frozen in liquid nitrogen, and stored at −80 °C for gene, enzyme, polyamine, and NO analysis. For CI evaluation, 20 fruits per replicate of each treatment were sampled weekly from cold storage and held at 20 °C for 3 days.

CI Evaluation. The CI index was individually evaluated in each fruit according to a previous paper,[13] with a four-stage scale based on the severity of the CI symptoms (surface pitting): 0 = no pitting; 1 = pitting covering <5% of the fruit surface; 2 = pitting covering <25% but >5% of surface; 3 = pitting covering <50% but >25% of surface, and 4 = pitting covering >50% of surface. The average extent of CI was expressed as a CI index, which was calculated using the following formula: CI index = $\Sigma[(\text{CI level}) \times (\text{number of fruits at the CI level})]/(\text{total number of fruits})$.

Electrolyte Leakage Assay. The rate of electrolyte leakage was determined as described by Zhao et al.[22] with modification. Disks (3 mm thick) of mesocarp tissue were excised with a 1 cm diameter stainless steel cork borer from the equatorial part of fruits. Six disks of each replicate were put into aqueous 0.1 M mannitol and shaken at 100 cycles/min for 2 h. The conductivity of the solution was measured with a conductivity meter (DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China). After readings were taken, the solutions were boiled for 10 min and then cooled to 20 °C. The conductivity was measured for total electrolytes. The rate of electrolyte leakage was expressed as a percentage of the total: (initial/total) $\times$ 100.

Quantitative Real-Time PCR (qRT-PCR) Assay. Total RNA was extracted by using the trizol method as described previously.[21] The potential contaminating genomic DNA was removed by digestion with DNase I (DNA-free; Ambion) according to the manufacturer's protocol. The cDNA was synthesized using oligo(dT)$_{18}$ and 2 μg of total RNA treated with RNase-free DNase I and M-MLV Reverse Transcriptase (Promega) according to the method of Zhao et al.[22] Transcript levels of each gene were evaluated via qRT-PCR using the SYBR Green I MasterMix (Toyobo, Osaka, Japan) on a Chromo4 real time PCR Detection System (Bio-Rad, Hercules, CA). Specific primers were designed from coding sequences of each gene using Primer Express 2.0 software (Applied Biosystems) (Table 1). *Ubi3* was used as the reference gene. The cDNA concentration used produced a cycle threshold (CT) between 15 and 30 cycles. The PCR amplification protocol consisted of a denaturation step at 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C and 20 s at 60 °C. The SYBR Green I fluorescence signal was measured during the 60 °C annealing step. To check the annealing

specificity of each oligonucleotide, melting curve analysis (55–94 °C) was carried out at the end of amplification. All experiments were run in triplicate with different cDNAs synthesized from three biological replicates. The relative gene expression for each sample was normalized to *Ubi3* mRNA and set relative to control samples (0 day) according to the $2^{-\Delta\Delta\text{CT}}$ method.

Arginase Assay. Arginase activity was determined according to the method of Chen et al.[23] with minor modifications. Frozen tissue (1 g) was extracted in 5 mL of 100 mM Tris-HCl (pH 7.5) containing 1% (v/v) β-mercaptoethanol, 0.1 mM phenylmethanesulfonyl fluoride (PMSF), and 0.5% (w/v) polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 12000g for 15 min at 4 °C, and the supernatants were used for the enzyme assays. The enzyme solution was made up to 1 mM in MnCl$_2$ and left for 15 min at 37 °C to activate arginase. Reaction mixtures contained 20 μL of the activated arginase, 420 μL of 120 mM glycine–NaOH (pH 9.6), 40 μL of 1.25 M arginine (pH 9.6), and 20 μL of 50 mM MnCl$_2$. Reactions were carried out at 37 °C for 20 min and stopped by adding 0.5 mL of 15% (v/v) perchloric acid. A 250 μL aliquot was mixed vigorously with 3 mL of acid mixture [27% (v/v) of sulfuric acid and 9% (v/v) of phosphoric acid] and 100 μL of 3% (w/v) α-isonitrosopropiophenone in 95% (v/v) ethanol. The mixtures were heated in a boiling water bath for 60 min in the dark. After the mixture had cooled to room temperature within 10 min, the absorbance was recorded at 520 nm, and urea standards were prepared for calibration. Arginase activity was expressed as nanomoles of urea produced per minute per milligram of protein.

ADC and ODC Assay. One gram of frozen tissue was homogenized in 5 mL of 100 mM potassium phosphate buffer at pH 6.3 containing 20 mM β-mercaptoethanol, 10 μM leupeptin, 5 mM ethylenediamine-tetraacetic acid (EDTA), and 100 μM pyridoxal phosphate. Homogenates were centrifuged at 20000g for 15 min at 4 °C. The supernatant was used as the enzyme source. The ADC and ODC activities were assayed according to the procedure described by Zhao et al.[24] One unit (U) of ADC or ODC activity was defined as the amount of enzyme that produced 1 μmol of agmatine or putrescine (Put) per minute at 37 °C, respectively. Activity was expressed as units per milligram of protein.

NOS Assay. Frozen samples (2 g) were ground to a fine powder in a mortar precooled with liquid nitrogen. The powder was mixed with 4 volumes of extraction buffer, which contained 50 mM phosphate-buffered saline (PBS, pH 7.4), 1.0 mM EDTA, 10.0 mM ethylene glycol bis(2-aminoethyl ether) tetraacetic acid, 1.0 mM leupeptin, 1.0 mM PMSF, and 1% PVP. The homogenate was centrifuged at 4 °C for 20 min at 15000g, and the supernatant solution was collected and used for assays.[25] The NOS activity was determined in the supernatant using an NOS colorimetric assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, People's Republic of China). Briefly, the supernatant was mixed with assay reagent containing arginine, and NOS in the specimen catalyzed arginine to citrulline and NO. NOS activity was indirectly determined by measuring NO production in a colorimetric assay. Absorbance was obtained at 530 nm against a blank prepared with distilled water to determine the concentration of nitrite, which is a NO metabolite. NOS activity was calculated with the formula according to the kit reference. One unit of enzyme activity was defined as the amount that catalyzed the formation of 1 nmol of NO from arginine per minute at 37 °C. The NOS activity was expressed as units per milligram of protein.

Protein Concentration. Protein concentration in the enzymatic extracts was quantified according to the method of Bradford[26] with bovine serum albumin as standard.

Free Polyamine Determination. Polyamine analyses were performed according to the method of Flores and Galston[27] with slight modifications. Fruit samples were homogenized in 4 volumes of 5% (w/v) cold perchloric acid and centrifuged at 20000g for 30 min at 4 °C. Aliquots (2 mL) of the supernatants were mixed with 2 mL of 2 M NaOH and 10 μL of benzoyl chloride. After vortexing for 20 s, the

Table 1. Genes and Oligonucleotides Used in the Quantitative Real-Time PCR Experiments

gene	GenBank accession no.	encoded proteins	primers (5′–3′)
LeARG1	AY656837	arginase	forward: GTGGAAAAAGGACAGAATCG
			reverse: AGAGACGTTGAGGCTACAGC
LeARG2	AY656838	arginase	forward: TGTTCGTGGACTTGGAGGTG
			reverse: CCCTGGAGAAATGAAGAGTTGTG
ADC	L16582	arginine decarboxylase	forward: GTGATCGTAAGGGCGGAAAG
			reverse: GCACGGGCATCTTCATTGAG
ODC	AF029349	ornithine decarboxylase	forward: AAACCCACTTCCACGACTTCC
			reverse: GACTCTTTTGCCGATGATGGTT
Ubi3	X58253	ubiquitin	forward: TCCATCTCGTGCTCCGTCT
			reverse: CTGAACCTTTCCAGTGTCATCAA

Table 2. Effects of MeSA Treatment on Chilling Injury (CI) Index and Electrolyte Leakage in Cherry Tomato Fruits during Storage at 2 °C for 28 Days[a]

days of storage	CI index		electrolyte leakage (%)	
	control	MeSA	control	MeSA
0			12.58 ± 1.08 dA	13.71 ± 0.36 dA
7	0.23 ± 0.03 d	ND	28.95 ± 1.78 cA	22.17 ± 1.29 cB
14	1.23 ± 0.08 cA	0.62 ± 0.06 cB	32.97 ± 4.70 cA	23.41 ± 2.85 cB
21	2.78 ± 0.28 bA	1.98 ± 0.12 bB	49.50 ± 0.62 bA	39.31 ± 4.14 bB
28	3.3 ± 0.15 aA	2.48 ± 0.10 aB	60.18 ± 2.03 aA	48.46 ± 3.70 aB

[a] Means in a column followed by different lower case letters and in a row followed by different capital letters for each mean are significantly different at $P = 0.05$ by Duncan's multiple-range tests. Data are accompanied by standard errors of the means. ND, not detected.

mixture was incubated for 20 min at 37 °C. The reaction was stopped by adding 2 mL of saturated NaCl solution. The benzoylated polyamines were then extracted with 2 mL of chilled diethyl ether and vortexed for 10 s. The ether phase (1 mL) was collected and dried under a stream of warm air. The residue was dissolved in 300 μL of methanol (HPLC grade) and filtered through 0.45 μm membrane filters. Aliquots (20 μL) were analyzed by HPLC using an Agilent 1200 series and a reverse phase C$_{18}$ column (Zorbax ODS, 5 μm particle diameter, 4.6 × 250 mm). Samples were eluted with 64% methanol at a flow rate of 1 mL min^{-1} and detected using a UV detector at 254 nm. Standard putrescine (Put), spermidine (Spd), and spermine (Spm) were subjected to the same procedure. The resulting values were compared with a standard curve constructed using known amounts of polyamines (Put, Spd, and Spm) and expressed as nanomoles of Put, Spd, or Spm per gram of fresh weight (FW).

NO Determination. NO was metabolized to nitrite and nitrate under acid conditions and quantitation of these stable anions using Griess reagent used to determine indirectly the amount of NO originally present. Extracts for NO were prepared according to the method of Hao et al.[26] with slight modifications. One gram of frozen samples was ground and homogenized in a mortar and pestle in 5 mL of cold buffer (50 mM PBS, 1 mM dithiothreitol, 1 mM MgCl$_2$, pH 7.4) and centrifuged at 10000g for 20 min. Supernatant solution was used to measure NO content according to the instruction of the nitric oxide assay kits (Nanjing Jiancheng Bioengineering Institute); absorbance was measured at 550 nm against a blank prepared with distilled water, and the release of NO into the culture medium was expressed as nitrite concentration, which was determined from a standard curve. NO content was expressed as micromolar per gram of FW.

Data Analysis. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) and Duncan's multiple comparisons were carried out to test any significant differences between the means. A probability of $P < 0.05$ was considered to be significant. All experiments were conducted in a completely randomized design with three replicates for each treatment. The data were expressed as the mean ± standard error (SE).

■ RESULTS

Effect of MeSA Treatment on Chilling Injury. Chilling symptoms appeared in control fruits on the first sampling day of cold storage and increased with storage time (Table 2). However, there were no obvious chilling symptoms in MeSA-treated fruits on day 7, and the CI index in MeSA-treated fruits was also lower than that of controls on days 14, 21, and 28 ($P < 0.05$). After 4 weeks at 2 °C, the CI index of control fruit was 26% higher than that of MeSA-treated fruit ($P < 0.05$).

Membrane damage, which could be reflected by electrolyte leakage, was one of the main results caused by CI. The change of electrolyte leakage shown in Table 2 exhibited a similar trend with CI index, which in both control and MeSA-treated fruits increased with storage time. Moreover, electrolyte leakage in control fruits was also significantly higher than that in MeSA-treated fruits at the same time of cold storage ($P < 0.05$).

Effect of MeSA Treatment on Gene Expression and Activity of Arginase. The expression patterns of the two genes
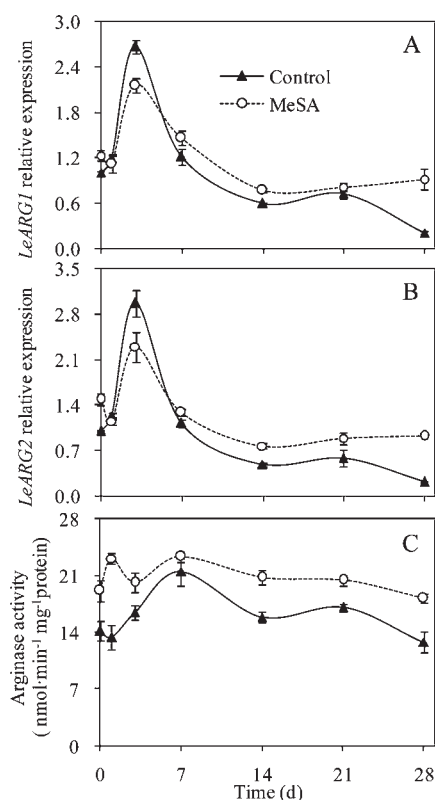
Figure 2. Gene expression and activity of arginase in cherry tomato fruit either untreated or treated with 0.05 mmol L^{-1} MeSA and stored at 2 °C for 28 days: expression of *LeARG1* (A) and *LeARG2* (B); arginase activity (C). The expression levels of *LeARG1* and *LeARG2* encoding arginase were evaluated by quantitative real-time PCR, normalized to the host *Ubi3* gene, and set relative to control sample from day 0 according to the $2^{-\Delta\Delta CT}$ method. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent standard errors of the means.

encoding arginase, *LeARG1* (Figure 2A) and *LeARG2* (Figure 2B), were very similar in control or MeSA-treated fruits during cold storage. The transcript levels of *LeARG1* and *LeARG2* increased as early as 1 day of storage, reaching a maximum level at day 3, and then decreased. Although the peak of expression levels of *LeARG1* and *LeARG2* during cold storage was decreased by MeSA treatment, both of them were induced immediately after MeSA treatment (day 0). The expression levels of *LeARG1* and *LeARG2* in fruits treated with MeSA were increased nearly 22 and 48% compared with those of the control fruit before cold storage (Figure 2A,B, day 0). In subsequent cold storage, the *LeARG1* expression level in MeSA-treated fruit was increased on days 7, 14, and 28, and the *LeARG2* expression level was also increased by MeSA treatment from day 7 to 28. Arginase, once synthesized, is relatively stable in plant tissue.[23] Therefore, enhanced expression levels of *LeARG* genes were accompanied by a large increase in arginase activity (Figure 2C). Before cold storage, arginase activity in MeSA-treated fruit was nearly 35% higher than that of the control. (Figure 2C, day 0). Moreover, during subsequent cold storage, arginase activity in MeSA-treated fruit was also higher than that of control except on day 7.

Effect of MeSA Treatment on Gene Expression and Activities of ADC and ODC. MeSA increased *ADC* and *ODC* expression immediately after treatment, with values nearly 241% and 150% higher than that of the control, respectively

(Figure 3A, day 0). In subsequent cold storage, the *ADC* expression level was higher in MeSA-treated fruit than in control except on day 1 (*P* < 0.05; Figure 3A). The *ODC* expression level was also enhanced by MeSA treatment during the storage periods except on days 1 and 7 (*P* < 0.05; Figure 3C).

Enhanced *ADC* expression was accompanied by an increase of 31% in ADC activity after fruit was exposed to MeSA vapor for 12 h (Figure 3B, day 0). Moreover, treatment with MeSA significantly increased ADC activity compared with the control during the subsequent cold storage period except on day 7. ODC activities were also induced immediately after exposure to MeSA vapor for 12 h (Figure 3D, day 0). In the subsequent cold storage, ODC activity in control and MeSA-treated fruits increased with some fluctuation and reached a maximum on day 14 (Figure 3C). Moreover, MeSA treatment increased ODC activity during the cold storage periods except on days 1 and 14 (*P* < 0.05).

Effect of MeSA Treatment on Free Polyamine Levels. The three main polyamines (Put, Spd, and Spm) in their free forms were identified and quantified, with Put being the major polyamine followed by Spd and Spm. The accumulation of Put was also observed in cherry tomato fruit under chilling stress, and MeSA treatment induced a larger increase in treated fruit than the control fruit throughout the cold storage period (Figure 4A). Spd and Spm levels were also increased by MeSA treatment before storage, being 24 and 28% higher than that of control fruits, respectively (Figure 4B,C, day 0) (*P* < 0.05). During cold storage, Spd levels in MeSA-treated fruits were also higher than that of control except on day 7 (*P* < 0.05). Although the levels of Spm decreased in response to MeSA treatment at the end of the experiment, Spm levels in MeSA-treated fruits were significantly higher than that of control from day 0 to day 21 (*P* < 0.05).

Effect of MeSA Treatment on NOS Activity and NO Content. As shown in Figure 5 (day 0), NOS activity and NO concentrations were 15 and 22% higher, respectively, immediately after than before MeSA treatment. Under chilling stress, NOS activity in control and treated fruits increased rapidly and reached a maximum on day 3. However, NOS activity in MeSA-treated fruit was significantly higher than that of control during the storage periods except on days 14 and 28 (*P* < 0.05). MeSA treatment also increased NO content during the storage periods except on days 14 and 21 (*P* < 0.05). By the end of cold storage, NO content in MeSA-treated fruit was 47% higher than that of control.

■ DISCUSSION

Arginase, ADC, and NOS are the three key enzymes in arginine catabolism in plants. Polyamines are synthesized through ADC and/or arginase−ODC pathway, whereas NO is synthesized via the NOS (Figure 1). In this study, MeSA treatment significantly induced the three pathways of arginine metabolism. Chen et al.[23] found that *LeARG* expression in tomato leaves increased rapidly in response to wounding and methyl jasmonate (MeJA) vapor treatment. Therefore, in our study, the levels of *LeARG1* and *LeARG2* expression were also detected after MeSA treatement for 3 and 6 h at 20 °C. Compared with control, *LeARG1* expression level increased 225 and 163% and *LeARG2* expression levels increased 233 and 338% in fruits treated with MeSA for 3 and 6 h at 20 °C, respectively (data not shown). After treatment with MeSA for 12 h at 20 °C, the expression levels of *LeARG1* and *LeARG2* in treated fruits were also higher than that in control fruits (Figure 2A,B, day 0). The expressions of *LeARG1* and *LeARG2*
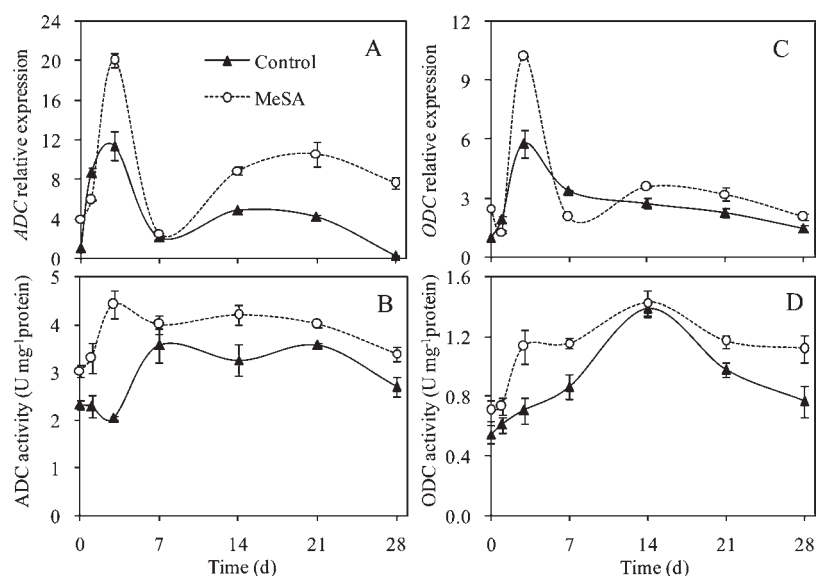
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Figure 3. Gene expression and activity of ADC and ODC in cherry tomato fruits either untreated or treated with 0.05 mmol L^{-1} MeSA and stored at 2 °C for 28 days.: *ADC* expression (A); ADC activity (B); *ODC* expression (C); ODC activity (D). The expression levels of *ADC* and *ODC* were evaluated by quantitative real-time PCR, normalized to the host *Ubi3* gene, and set relative to control sample from day 0 according to the $2^{-\Delta\Delta CT}$ method. Data are expressed as the mean $\pm$ SE of triplicate assays. Vertical bars represent standard errors of the means.
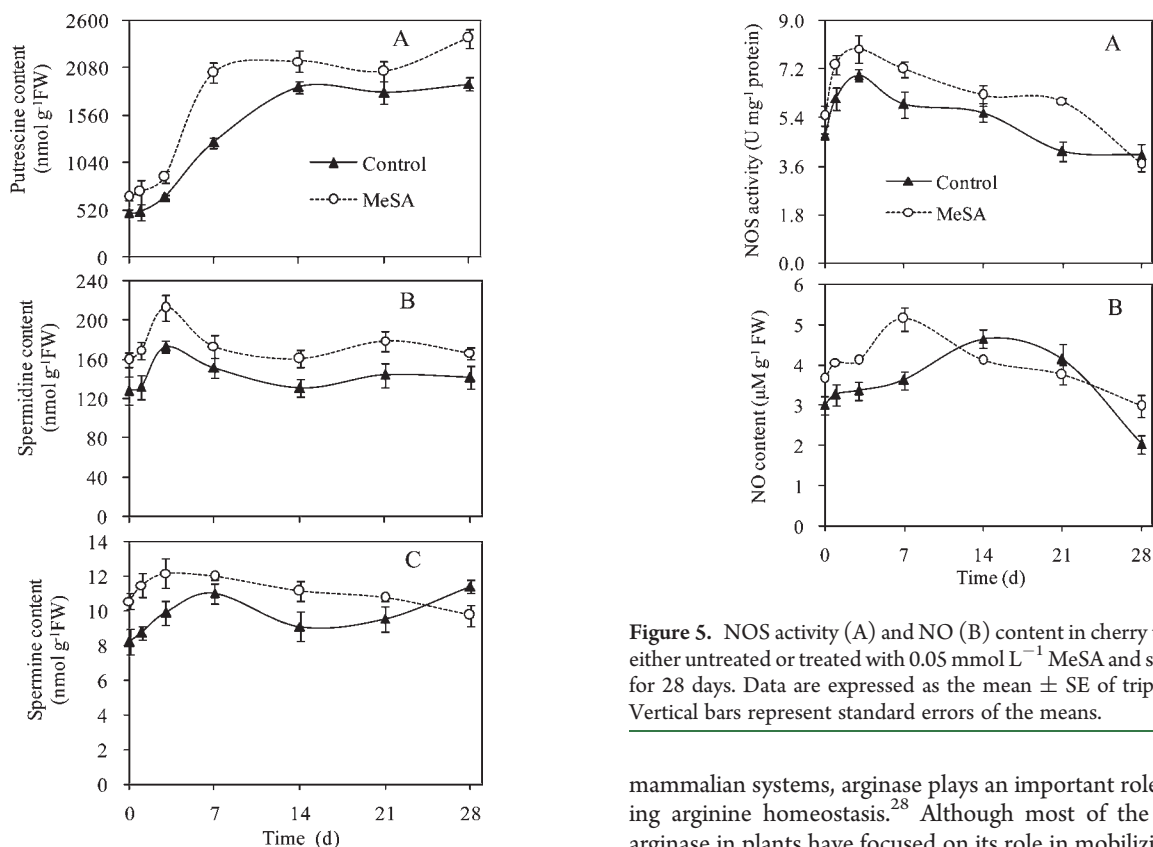

Figure 4. Concentrations of putrescine (A), spermidine (B), and spermine (C) in cherry tomato fruits either untreated or treated with 0.05 mmol L^{-1} MeSA and stored at 2 °C for 28 days. Vertical bars represent standard errors of the means (n = 3).

were accompanied by an obvious increase in arginase activity in fruits treated with MeSA throughout the storage periods.
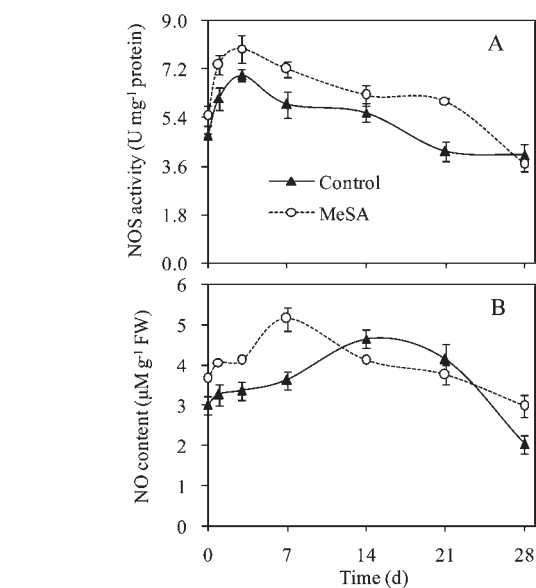

Figure 5. NOS activity (A) and NO (B) content in cherry tomato fruits either untreated or treated with 0.05 mmol L^{-1} MeSA and stored at 2 °C for 28 days. Data are expressed as the mean $\pm$ SE of triplicate assays. Vertical bars represent standard errors of the means.

mammalian systems, arginase plays an important role in regulating arginine homeostasis.[28] Although most of the studies on arginase in plants have focused on its role in mobilizing arginine during early seedling germination, plant arginase was also reported to be involved in stress responses. Arginase gene expression and activity in the tissues of tomato and *Arabidopsis* increased in response to wounding, disease, MeJA, and low temperature.[2,21,23] Brownfield et al.[29] reported that *ARGAH2* was required for normal induction of At2g1461, a pathogenesis response family member, following MeJA treatment. Moreover,

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in a previous study, we found that inhibition of arginase activity could aggravate CI in cherry tomato fruits.[21] Therefore, it is reasonable to suggest that the elevated arginase activity in treated fruit may be involved in the chilling tolerance induced by MeSA treatment.

Polyamines are the major metabolites of arginine, high levels of which have been correlated with increased chilling resistance in many horticultural crops.[17,20] It has been hypothesized that polyamines protect the integrity of membranes and in turn alleviate CI.[17] In plants, polyamines are synthesized by the decarboxylation of arginine or ornithine catalyzed by ADC or ODC, respectively.[17] Some papers support that ADC- and ODC-mediated Put biosynthesis pathways play different roles in plant development and growth. It seems that ADC is related to tissue maturation and response to environmental stresses, whereas ODC plays the main role in cell division.[17,30] However, in our present study, we found that MeSA treatment significantly induced the gene expression levels and activities of both ADC and ODC, which would contribute to polyamine accumulation. Németh et al.[31] reported that exogenous SA treatment caused a significant increase in Put and Spd content in the leaves of maize under cold stress. Cao et al.[32] found that the combination of heat and SA treatment increased the polyamine concentrations and effectively alleviated the CI of peach fruit. Our present study found that MeSA promoted the catabolism of arginine and increased polyamine levels. The reduction in CI of cherry tomato fruits by MeSA treatment in our study may in part be due to increased polyamine concentrations.

In animal systems most of the NO produced is due to NOS activity, which catalyzes the oxygen- and NADPH-dependent oxidation of arginine to NO and citrulline in a complex reaction.[4] In plants, mammalian-like NOS activities have already been detected, and mammalian NOS inhibitors also effectively block NO production.[3,8,9] Recently, Zheng et al.[7] also found that NOS is a key enzyme regulating NO production in induced resistance of tomato fruit. In the present investigation, MeSA treatment resulted in an increase in NOS activity and NO content in cherry tomato fruits (Figure 5). Despite the fact that NO can also be generated by other enzymes apart from NOS and nonenzymatic mechanisms in plant,[16] the accumulation of NO in cherry tomato fruit induced by MeSA treatment was, at least in part, from the NOS pathways. A similar result was also reported by Zottini et al.,[9] who found that NO was a downstream signal in the SA-induced response in *Arabidopsis* and that NO production proceeds mainly through a NOS-dependent pathway.

In plants, NO is established as a multifunctional signaling molecule involved in many physiological processes as well as an important messenger for induced defense responses.[16] In recent research, NO has been applied to reduce the development of CI symptoms in a number of horticultural crops.[18,19] These researchers suggested that NO was also involved in the chilling tolerance of horticultural crops. From these results and the fact that fruit treated with MeSA showed a lower sensitivity to chilling, it cannot be ruled out that the tolerance of cherry tomato fruit induced by MeSA treatment may be partly related to the activated NOS pathways of arginine catabolism.

In conclusion, our results indicate that the coordinated activation of different pathways of arginine catabolism may be involved in the chilling tolerance of cherry tomato fruits induced by MeSA. The gene expression and activities of ADC, arginase, and ODC were enhanced by MeSA treatment, which resulted in accumulation of endogenous polyamine levels. In addition, NO content in MeSA-treated fruit was increased compared with that of control fruit, which was partly due to the increased NOS activity. The reduction in CI by MeSA treatment may be due to the increase in polyamine and NO levels, which is primarily due to the activated arginine catabolism. To our knowledge, this is the first evidence of arginine catabolism involved in chilling tolerance of a postharvest horticultural product, which may provide a new perspective for the mode of MeSA action in inducing chilling tolerance of plants.

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■ ABBREVIATIONS USED

MeSA, methyl salicylate; CI, chilling injury; SA, salicylic acid; NO, nitric oxide; ADC, arginine decarboxylase; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; qRT-PCR, real-time PCR; CT, cycle threshold; PMSF, phenylmethanesulfonyl fluoride; PVP, polyvinyl polypyrrolidone; EDTA, ethylenediaminetetraacetic acid; FW, fresh weight; PBS, phosphate-buffered saline; MeJA, methyl jasmonate; Put, putrescine; Spd, spermidine; Spm, spermine.

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