Involvement of Arginase in Methyl Jasmonate–induced Tomato Fruit Chilling Tolerance

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ABSTRACT. The physiological role of arginase in nitrogen remobilization processes from protein degradation during seed germination has well been described in several species. However, very little is known about its possible roles in plant stress responses. Treatment of tomato fruit (*Solanum lycopersicum* L.) with 0.05 mM methyl jasmonate (MeJA) enhanced transcription levels of arginase genes, especially *LeARG2*. Chilling injury (CI) of fruit treated with 0.05 mM MeJA for 12 hours was also effectively alleviated, as manifested by decreases in CI index, electrolyte leakage, and malondialdehyde (MDA) content. To investigate the potential role of arginase in MeJA-induced chilling tolerance, fruit were treated with MeJA or the arginase inhibitor N^ω-hydroxy-nor-L-arginine (nor-NOHA) combined with MeJA and then stored at 2 °C for 28 days. MeJA-induced arginase activity was strongly inhibited and the reduction of CI by MeJA was nearly abolished by the inhibitor. In addition, MeJA treatment increased the activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX); inhibited peroxidase (POD) activities; and promoted proline and polyamines accumulation. These effects were partially counteracted by nor-NOHA; putrescine accumulation, however, was unaffected by the inhibitor. Our results indicate that arginase may be involved in MeJA-induced chilling tolerance, possibly by ameliorating the antioxidant enzyme system of fruit and increasing proline levels.

Arginine is one of the most versatile amino acids in living cells. In addition to serving as a building block of proteins, arginine can positively modulate several subsequent ornithine-dependent pathways through its catabolism (Gao et al., 2009; Jubault et al., 2008). Arginase hydrolyzes arginine to urea and ornithine, the latter of which contributes to the biosynthesis of both polyamines and proline (Jubault et al., 2008). Research on the role of arginase in stress responses in plants has gained considerable attention in recent years. Brownfield et al. (2008) reported that *ARGAH2* was required for normal induction of At2g14610 following MeJA treatment in arabidopsis [*Arabidopsis thaliana* (L.) Heynh.] tissues. At2g14610 is annotated as a pathogenesis response 1 family member. Increased expression of arginase upon stress imposition has been observed (Brauc et al., 2011; Chen et al., 2004; Jubault et al., 2008), and silencing *ARGAH2* resulted in enhanced gall sizes in infected arabidopsis tissues (Gravot et al., 2012). In addition, over-expression of arginase was accompanied by an increased resistance to phytophagous insects in tomato (Chen et al., 2005). In our previous study, the arginase activity and expressions of both *LeARG1* and *LeARG2* increased in tomato fruit during cold storage; these changes increased the chilling tolerance of fruit (Zhang et al., 2010).

CI is a physiological defect of many tropical and subtropical horticultural products that results in reduced quality and loss of product utilization following exposure to low but nonfreezing temperatures. MeJA, a natural plant growth regulator, plays important roles in plant growth and development, fruit ripening, and responses to environmental stress (Wasternack, 2007). Treatment with MeJA at nontoxic concentrations can alleviate CI symptoms in a number of horticultural crops (Cao et al., 2009; Meng et al., 2009). Thus, MeJA treatment may potentially be used commercially for alleviating CI in fruit and vegetables. However, the mode of action of MeJA in reducing CI and quality deterioration remains incompletely understood.

Tomato fruit, a typical chilling-sensitive horticulture crop, is an excellent model system for investigating the physiological and molecular mechanisms of postharvest CI in fruit during cold storage. In the present study, we found that treatment of tomato fruit with MeJA results in strong induction of *LeARG2*, a second gene encoding arginase. Enhanced expression of the arginase gene in response to MeJA has also been observed in arabidopsis; this activity was reported to be a MeJA-driven defense mechanism against stress by the regulation of the arginine-derived polyamines or proline content (Brownfield et al., 2008). The involvement of polyamines and proline in chilling stress responses has been extensively studied. An increase in polyamines or proline levels is correlated with improved cold tolerance in chilling-sensitive plants (Cao et al., 2012; Mirdehghan et al., 2007). However, the possible role of arginase induction response to MeJA during the cold storage of

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tomato fruit and its consequences on primary metabolism and ultimately the CI process remain to be clarified. The objective of this study was to investigate the role of arginase in MeJA-induced chilling tolerance by applying nor-NOHA (an ideal inhibitor of arginase) to tomato fruit.

Materials and Methods

PLANT MATERIAL AND TREATMENTS. Tomato fruit, free from blemishes or disease, were hand-harvested at the mature green stage from an orchard in Shandong, People's Republic of China. All of the fruit were selected for uniformity of shape, color (green), and size. Fruit treatments were divided into two phases according to the experimental methodology. In the first phase, fruits (≈ 100) were randomly divided into 10 lots. Each lot was placed in a 9-L airtight container, together with 98 μ L of pure MeJA (Sigma-Aldrich, St. Louis, MO) or water (control) spotted onto filter paper and incubated for 0, 3, 6, 12, or 24 h in darkness at 20 °C (Fig. 1). After treatment, fruit were removed for gene expression analysis. Three replicates of each experiment were conducted. In the second phase, fruit were randomly divided into three lots of 330 fruit each for the following treatments in three replicates. One lot was dipped in 30 μ M nor-NOHA in a stainless steel vacuum container and vacuum-infiltrated under low pressure (-35 kPa) for 0.5 min; thereafter, fruit were kept under ambient air pressure for 2 min and then air-dried. The two other lots were first treated with water under the same conditions, after which half of the fruit were treated with 490 μ L of pure MeJA in a 45-L sealed chamber at 20 °C for 12 h and the other half were subjected to the same condition without exposure to MeJA; this half served as the control lot. For the combination nor-NOHA + MeJA treatment, fruit pretreated with nor-NOHA were also treated with 490 μ L of pure MeJA in a 45-L sealed chamber at 20 °C for 12 h. Following treatment, the chambers were opened, and three lots of fruit were stored at 2 ± 1 °C with a relative humidity of 80% to 90% for up to 28 d.

Mesocarp tissue was collected from the equatorial surface of 10 fruit per replicate at different hours or days for gene, enzyme, electrolyte leakage, MDA, polyamines, and proline analyses. Another sample of 15 fruit was removed weekly from cold storage and held at 20 °C for 3 d to simulate shelf conditions for CI evaluation.

RNA EXTRACTION AND QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION (qPCR) ASSAY. Total RNA was isolated from each sample using Trizol reagent following the user manual. Complementary DNA (cDNA) was synthesized from 2 μ g of

deoxyribonuclease-treated RNA and oligo(dT)15 primer with *Moloney murine leukemia virus* reverse transcriptase (Promega, Madison, WI). The synthesized cDNA was used for a qPCR analysis using the SYBR Green I MasterMix (Toyobo, Osaka, Japan) on a Chromo4 real-time PCR Detection System (Bio-Rad, Hercules, CA). The ubiquitin gene (*Ubi3*) was used as the reference gene. Nucleotide sequences of specific primers were as reported previously (Zhang et al., 2011). The qPCR reaction parameters were the following: 2 min at 95 °C and then 40 cycles of 95 °C for 15 s and 60 °C for 20 s. The SYBR Green I fluorescence signal was picked up during the annealing step at 60 °C. To control the specificity of each oligonucleotide, melting curve analysis (55 to 94 °C) was carried out at the end of amplification protocol. To determine the relative *LeARGs* transcript levels among all samples, the threshold cycle (Ct) value was normalized to *Ubi3* Ct value and calculated using the equation $2^{-\Delta\Delta C_t}$.

CI INDEX EVALUATION. CI was evaluated based on the percentage of affected surface area by sheet pitting; 0 = no injury; 1 = <5% of the surface; 2 = 5% to 25% of the surface; 3 = 25% to 50% of the surface, and 4 = >50% of the surface. The CI index was calculated as $\sum(CI \text{ level} \times \text{number of fruit at the CI level}) / (\text{total number of fruit} \times 4)$.

ELECTROLYTE LEAKAGE AND MDA CONTENT ASSAYS. The electrolyte leakage rate was measured as described previously (Zhao et al., 2009). Six mesocarp discs of each replicate were rinsed and incubated in 100 mL of 0.1 M mannitol for 2 h under constant shaking, and then the initial electrolyte leakage was measured. The solution was boiled for 10 min to completely kill the tissues. After cooling to room temperature, the total conductivity was then measured. The electrolyte leakage rate was defined as a percentage of the total: $(\text{initial}/\text{total}) \times 100$.

The content of MDA was measured following the method of Zhao et al. (2009). Tissue samples (2 g) were homogenized in 6.0 mL of 10% (w/v) trichloroacetic acid and centrifuged at 10,000 g_n for 20 min. The supernatant was mixed with 3 mL of 0.6% (v/v) thiobarbituric acid, heated at 100 °C for 30 min and then immediately cooled on ice. After centrifugation at 5000 g_n for 10 min, absorbance of the reaction product at 532 nm was recorded and corrected with nonspecific absorbance at 600 nm. The amount of MDA was expressed as micromoles per gram fresh weight (FW).

ARGINASE ASSAYS. Frozen tissue (1 g) was homogenized with 5 mL of 100 mM Tris-HCl (pH 7.5) containing 1% (v/v) β -mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, and 0.5% (w/v) polyvinylpyrrolidone. Homogenates were centrifuged at 12,000 g_n for 15 min at 4 °C, and the supernatants were used as the enzyme source. Before assays, the enzyme extract was activated with 1 mM $MnCl_2$ at 37 °C for 15 min. The reaction mixture contained 20 μ L of the activated enzyme source in assay buffer [120 mM glycine-NaOH buffer (pH 9.6), 100 mM L-arginine, 2 mM $MnCl_2$]. Reactions were performed at 37 °C for 20 min and stopped by addition of 0.5 mL of 15% (v/v) perchloric acid. The

Fig. 1. Schematic diagram for tomato fruit treatment with methyl jasmonate (MeJA). Ten fruit were placed in a 9-L airtight container, together with 98 μ L of pure MeJA or water (control) spotted onto filter paper, and incubated for 0, 3, 6, 12, or 24 h in darkness at 20 °C for the analysis of the expression pattern of arginase genes in response to MeJA treatment.

released urea in the medium was mixed with 3% (w/v) α -isonitrosopropiophenone in 95% (v/v) ethanol and acid mixture [27% (v/v) of sulfuric acid and 9% (v/v) of phosphoric acid]. This mixture was heated for 60 min in a boiling water bath in the dark. After cooling to room temperature within 10 min, the optical density of the supernatant was determined at 540 nm (Chen et al., 2004). Urea standards were prepared for calibration. Arginase activity was expressed as nanomoles of urea produced per minute per milligram of protein.

ANTIOXIDANT ENZYME ASSAYS. Frozen tissue (2 g) was homogenized with 8 mL of cold 50 mM sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 g_n for 15 min at 4 °C. The supernatants were used for the enzyme assays.

SOD activity was assayed according to Rao et al. (1996). SOD estimation was based on its ability to inhibit the photochemical reduction of nitrotriazolium blue chloride at 560 nm and is expressed as units per milligram protein.

CAT activity was assayed by monitoring the initial rate of hydrogen peroxide (H_2O_2) disappearance at 240 nm according to the method of Chance and Maehly (1955). One unit of CAT activity was defined as the amount of enzyme that decomposed 1 μ mol H_2O_2 per minute at 30 °C.

APX activity was assayed from the decrease in absorbance at 290 nm as ascorbate oxidized (Nakano and Asada, 1981). One unit of enzyme activity was defined as the amount of enzyme that oxidized 1 μ mol ascorbate per minute at 30 °C.

POD activity was determined as described by Chance and Maehly (1955). Briefly, the enzyme activity was assayed spectrophotometrically at 470 nm in a cuvette containing 50 mM sodium acetate (pH 5.0), 0.1% (v/v) H_2O_2 , 0.1% (w/v) guaiacol, and crude enzyme extract. One unit of enzyme activity was defined as the amount of enzyme causing a change of 0.01 absorbance per minute.

PROTEIN CONCENTRATION. Soluble protein content in the enzymatic extracts was determined according to the method of Bradford (1976) using bovine serum albumin as a standard.

PROLINE ASSAYS. Proline content was determined using the acid ninhydrin method described by Cao et al. (2012) with modification. Frozen samples of 2 g were homogenized in 6 mL 3% (v/v) sulfosalicylic acid at 100 °C for 10 min with shaking. The supernatant was treated with ninhydrin dissolved in acetic acid, boiled for 30 min. After cooling, the mixture was extracted with toluene and the OD₅₂₀ of the toluene layer was read at 520 nm.

FREE POLYAMINE DETERMINATION. Polyamines were assayed following Flores and Galston (1982). In brief, 1 g of fruit tissue was extracted with 5% (w/v) perchloric acid at 0 °C for 30 min. After centrifugation, 2 mL of the supernatant was mixed with 2 mL of 2 M NaOH and 10 μ L of benzoyl chloride. After vortexing for 20 s, the mixture was incubated for 20 min at 37 °C. The reaction was stopped by addition of 2 mL of saturated NaCl solution. The benzoylated polyamines were then extracted from the aqueous phase with diethyl ether. The ether phase was collected and evaporated to dryness. The residue was dissolved in 300 μ L of methanol, and the polyamines were separated by high-performance liquid chromatography (600E; Waters, Milford, MA). The benzoylated polyamines were eluted with 64% (v/v) methanol on a reverse-phase column (symmetry octadecyl, 5 μ m; 250 $\times$ 4.6 mm) at 30 °C and monitored with an ultraviolet detector at 254 nm. Different polyamines were quantified

based on the retention time and calibration curves with benzoylated standards.

STATISTICAL ANALYSIS. Experiments were conducted in a completely randomized design. Statistical analyses were performed with SPSS 16.0 (IBM Corp., Armonk, NY) statistical software. Data were analyzed by one-way analysis of variance. The mean values were compared by Duncan's multiple range tests, and the differences were considered significant at $P \leq 0.05$.

Results

TRANSCRIPT LEVELS OF ARGINASE IN RESPONSE TO MeJA TREATMENT. The expression of the two genes encoding arginase, *LeARG1* and *LeARG2*, in control fruit remained unchanged at 20 °C for 24 h (Fig. 2A and B), whereas treatment of fruit with MeJA resulted in strong induction of *LeARG2* (Fig. 2B). *LeARG2* transcripts increased rapidly within 3 h of MeJA treatment and increased with the exposure time. After 24 h of exposure to MeJA, the expression levels of *LeARG2* were $\approx$ 23-fold higher than that in control fruit. *LeARG1* expression also increased in MeJA-treated fruit (Fig. 2A), but was only 10% of the *LeARG2* mRNA levels after exposure to MeJA for 24 h.

MeJA-INDUCED ARGINASE ACTIVITY IS INHIBITED BY NOR-NOHA DURING COLD STORAGE. Arginase activity in control fruit increased in response to chilling stress, reaching a peak on day 7, and then decreased gradually (Fig. 3). Treatment with MeJA significantly enhanced arginase activity during the entire storage period. As expected, the inductive effect of MeJA on

Fig. 2. Relative expression of (A) *LeARG1* and (B) *LeARG2* in tomato fruit after treatment with 0.05 mM methyl jasmonate (MeJA) for various times. The expression levels of *LeARG1* and *LeARG2* encoding arginase were evaluated by quantitative real-time polymerase chain reaction, normalized to *Ubi3* gene and set relative to control samples from 0 h according to the $2^{-\Delta\Delta C_t}$ method. Different letters atop bars indicate significant differences among treatments and exposure durations at $P \leq 0.05$ by Duncan's multiple range test. Values are mean $\pm$ SE of triplicate assays.

Fig. 3. Effect of methyl jasmonate (MeJA) treatment on arginase activity in tomato fruit during storage at 2 °C with or without pretreatment by arginase inhibitor N^ω-hydroxy-nor-L-arginine (nor-NOHA). Different letters among treatments for the same storage time indicate significant differences at $P \leq 0.05$ by Duncan's multiple range test. Values are mean $\pm$ SE of triplicate assays.

arginase activity was prevented by treatment of fruit with nor-NOHA, a highly specific inhibitor of arginase, before MeJA treatment. In contrast to the control fruit, no significant difference in arginase activity was observed in (nor-NOHA + MeJA)-treated fruit throughout the cold storage periods.

CHANGES IN CI. No significant difference in CI was observed among all treatments on the first sampling day, as indicated by the similar CI indices, electrolyte leakage, and MDA contents (Fig. 4). However, MeJA treatment significantly inhibited the development of CI symptoms after 2 weeks of cold storage. As shown in Fig. 4A, the CI index of fruit treated with MeJA was 22.7% and 15.5% lower than those of the control and (nor-NOHA + MeJA)-treated fruit, respectively, at the end of storage. The electrolyte leakage and MDA content in MeJA-treated fruit were also lower than those obtained in other treatments (Fig. 4B and C). However, the inductive effects of MeJA on chilling tolerance were inhibited by treatment fruit with nor-NOHA before exposure to MeJA. During the entire storage periods, no significant differences in CI index, electrolyte leakage, and MDA contents between controls and (nor-NOHA+HA)-treated fruit were observed.

CHANGES IN ACTIVITIES OF ANTIOXIDANT ENZYMES. SOD, CAT, and APX activities increased significantly in MeJA-treated fruit compared with controls during most of the storage periods (Fig. 5A–C). At the end of storage, SOD, CAT, and APX activities in MeJA-treated fruit were 57.7%, 32.8%, and 32.9% higher, respectively, than those in the controls. The MeJA-induced activities of SOD, CAT, and APX were partially inhibited by exposure of the fruit to nor-NOHA before MeJA treatment. No significant differences in the activities of these enzymes between (nor-NOHA+MeJA)-treated and control fruit were observed after storage for 28 d. On the contrary, POD activity was minimal in MeJA-treated fruit and the combination of nor-NHOHA and MeJA treatment had little effect on POD activity during the entire storage periods (Fig. 5D).

CHANGES IN THE CONTENT OF PROLINE. As shown in Table 1, accumulation of proline was observed in control fruit under chilling stress, reaching a maximum on day 21. A similar tendency in proline content was also found in MeJA-treated

Fig. 4. Effect of methyl jasmonate (MeJA) treatment on (A) CI index, (B) electrolyte leakage, and (C) malondialdehyde (MDA) content in tomato fruit during storage at 2 °C with or without arginase inhibitor N^ω-hydroxy-nor-L-arginine (nor-NOHA) pretreatment. Chilling injury (CI) was evaluated based on the percentage of affected surface area by sheet pitting; 0 = no injury, 1 = <5% of the surface, 2 = 5% to 25% of the surface, 3 = 25% to 50% of the surface, and 4 = >50% of the surface. The CI index was calculated as $\sum(\text{CI level} \times \text{number of fruit at the CI level}) / (\text{total number of fruit} \times 4)$. Different letters among treatments for the same storage time indicate significant differences at $P \leq 0.05$ by Duncan's multiple range test. Values are mean $\pm$ SE of triplicate assays.

fruit, and proline contents in MeJA-treated fruit were significantly higher than those in controls on days 21 and 28. However, the MeJA-induced accumulation of proline was prevented by nor-NOHA. No significant differences in proline content were observed between controls and (nor-NOHA + MeJA)-treated fruit during the entire cold storage periods except on day 14.

CHANGES IN THE CONTENT OF POLYAMINES. Putrescine was the predominant polyamines in tomato fruit, the content of which increased by MeJA treatment on sampling days 7 and 28 (Table 1). A similar effect of MeJA was also found on spermidine content. Free spermine contents are relatively low in tomato fruit. However, spermine levels increased significantly in MeJA-treated fruit compared with controls during the entire

storage periods expect on day 28. Nor-NOHA treatment exerted limited effects on MeJA-induced putrescine and spermine accumulation, while it inhibited MeJA-induced spermidine contents.

Discussion

Research on arginase in mammals has gained considerable attention in recent years due to its function in a very wide range of physiological and pathophysiological conditions (Morris,

2009). Although the primary function of plant arginase has traditionally been perceived to be limited to the nitrogen remobilization processes from protein degradation, especially during seed germination (Goldraj and Polacco, 2000; King and Gifford, 1997), arginase induction is reported to be part of a defense mechanism against biotic and abiotic stress (Brauc et al., 2011; Brownfield et al., 2008; Chen et al., 2005; Gravot et al., 2012). In our present study, we found that MeJA treatment not only resulted in strong induction of *LeARGs*, especially *LeARG2* (Fig. 2B), which lead to increased arginase

activity in tomato fruit during cold stress (Fig. 3), but also effectively reduced fruit CI symptoms (Fig. 4A–C). Accumulation of transcripts of *ARGAH2* with an increase in arginase activity in response to MeJA has also been observed in arabidopsis, which indicates that arginase was involved in the defense responses induced by MeJA signaling (Brownfield et al., 2008). Induction of *LeARG2* and arginase activity in response to MeJA was previously reported in tomato by Chen et al. (2004). To investigate the role of arginase in MeJA-induced chilling tolerance, fruit were incubated with the arginase inhibitor nor-NOHA before treatment with MeJA. Compared with MeJA-treated fruit, fruit treated with nor-NOHA+MeJA showed a significantly higher CI index, as well as increased electrolyte leakage and MDA contents. The results presented here indicate that the expression of arginase may be involved in the chilling tolerance induced by MeJA.

The coordinated action of antioxidant enzymes such as SOD, APX, CAT, and POD is very important for scavenging active oxygen species to

Fig. 5. Effect of methyl jasmonate (MeJA) treatment on superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) activities in tomato fruit during storage at 2 °C with or without pretreatment by arginase inhibitor N^ω-hydroxy-nor-L-arginine (nor-NOHA). Different letters among treatments for the same storage time indicate significant differences at *P* ≤ 0.05 by Duncan's multiple range test. Values are mean ± SE of triplicate assays.

Table 1. Effect of methyl jasmonate (MeJA) treatment on proline, putrescine, spermidine, and spermine contents in tomato fruit during storage at 2 °C with or without pretreatment by arginase inhibitor N^ω-hydroxy-nor-L-arginine (nor-NOHA).

Storage time (d)	Treatment	Proline	Putrescine	Spermidine	Spermine
		[mean ± SE (nmol·g ⁻¹ FW)]			
7	Control	207.40 ± 18.67 a ^z	1,230.35 ± 70.40 b	151.70 ± 10.05 b	11.01 ± 0.59 b
	MeJA	215.96 ± 10.13 a	1,617.51 ± 132.44 a	175.87 ± 8.66 a	12.80 ± 0.86 a
	nor-NOHA + MeJA	208.14 ± 2.33 a	1,482.14 ± 118.97 a	155.29 ± 10.19 b	11.77 ± 0.69 ab
14	Control	236.62 ± 16.19 a	1,841.05 ± 67.37 a	131.22 ± 13.76 a	9.09 ± 0.86 c
	MeJA	253.85 ± 14.48 a	1,891.05 ± 112.69 a	137.03 ± 3.33 a	13.36 ± 0.57 a
	nor-NOHA + MeJA	189.59 ± 18.27 b	1,834.77 ± 109.03 a	143.21 ± 5.66 a	11.76 ± 0.65 b
21	Control	258.71 ± 10.68 b	1,808.55 ± 57.71 a	144.14 ± 12.09 a	9.55 ± 0.72 b
	MeJA	297.41 ± 15.15 a	1,725.13 ± 110.33 a	139.03 ± 15.00 a	11.49 ± 0.47 a
	nor-NOHA + MeJA	239.45 ± 10.15 b	1,961.32 ± 149.5 a	131.38 ± 13.95 a	10.92 ± 0.93 ab
28	Control	211.45 ± 10.15 b	1,900.39 ± 88.98 b	136.92 ± 12.27 b	10.28 ± 0.38 a
	MeJA	261.24 ± 14.17 a	2,383.43 ± 99.79 a	168.50 ± 11.22 a	11.17 ± 0.57 a
	nor-NOHA + MeJA	229.35 ± 9.16 b	2,238.12 ± 127.12 a	147.18 ± 6.48 b	11.38 ± 0.66 a

^zNumbers in a column not followed by the same letter for the same storage time are statistically different using Duncan's multiple range test at *P* ≤ 0.05.

protect cell membranes (Cao et al., 2009; Sevillano et al., 2009). Evidence shows that higher activities of SOD, CAT, and APX are positively correlated with chilling tolerance in post-harvest horticultural crops (Cao et al., 2009; Jin et al., 2009). Our results also confirmed these findings since we found that the activities of SOD, CAT, and APX increased in MeJA-treated fruit along with the alleviation of CI symptoms under chilling stress (Fig. 5A–C). However, MeJA treatment exerted negative effects on POD activity (Fig. 5D). POD activity in (nor-NOHA + MeJA)-treated fruit was higher than that in MeJA-treated fruit. An alleviation of CI associated with an increase of POD activity in horticultural crops under chilling stress has also been reported (Jin et al., 2009; Safizadeh et al., 2007). The current results as well as those reported in prior studies suggest that the balance between the activities of SOD and subsequent H_2O_2 scavenging enzymes is critical to cell survival during cold storage. It is well known that SOD catalyzes the dismutation of superoxides to H_2O_2 and that H_2O_2 can be destroyed by CAT, APX, or POD. However, the mode of action of POD differs from CAT in that POD liberates free radicals rather than oxygen (Safizadeh et al., 2007). In our study, MeJA treatment decreased POD activity and increased CAT and APX activities, which not only scavenge H_2O_2 but also alleviate the production of phytotoxic free radicals liberated by POD. However, the coordinated action of these enzymes in MeJA-treated fruit was diminished upon inhibition of arginase activity by nor-NOHA, which suggests that MeJA alters the activities of these enzymes at least in part through the induction of arginase. Arginase catalyzes the conversion of arginine to ornithine and ornithine can further feed into the important downstream pathways of polyamine and proline syntheses. Our data showed that MeJA treatment not only increased arginase activity but also significantly promoted proline accumulation (Table 1). In contrast and as expected, levels of proline markedly decreased in fruit treated with nor-NOHA + MeJA, which indicated that the metabolic flux from arginine to proline was attenuated by inhibiting arginase activity. Therefore, the MeJA-induced proline accumulation in tomato fruit may be due to the activation of arginase. Increases in arginase activity or expression of *ARGAH2* gene along with an increase in proline content have been reported in arabidopsis (Brauc et al., 2011; Jubault et al., 2008). Increases in proline content in several horticultural crops, such as tomato (Zhao et al., 2009), peach [*Prunus persica* L. (Shang et al., 2011)], and loquat [*Eriobotrya japonica* L. (Cao et al., 2012)] fruit, are known to be associated with increased resistance to chilling stress during cold storage. Mohammadrezakhani and Pakkish (2015) also reported that grapes (*Vitis vinifera* L.) treated with proline significantly increased SOD, CAT, and APX activity. In the present study, therefore, inhibiting arginase activity using nor-NOHA abolished MeJA-induced activities of antioxidant enzymes, suggesting that may be related to the decrease of proline levels. At low temperatures, proline accumulation plays a major role in antioxidative stress as a hydroxyl radical scavenger, osmotic regulation, and protein-comptible hydrotrope (Delauney and Verma, 1993). Our data suggest that arginase may be involved in MeJA-induced chilling tolerance of tomato fruit partly by promoting the formation of proline.

Polyamines are regarded as a new type of plant growth regulators that are purportedly involved in a broad spectrum of physiological processes and stress responses (Groppa and

Benavides, 2008). In fruit, most of the available research work has focused on the role of polyamines in CI development, wherein polyamines, especially putrescine, tends to accumulate in plant organs exposed to chilling stress. Evidence of the effectiveness of conditioning treatments for reducing chilling symptoms by increasing polyamines has also been obtained (Mirdehghan et al., 2007; Zhang et al., 2011). However, Valero et al. (2002) pointed out that whether increases in polyamines levels were a protective mechanism against CI or simply a tissue response to this stress was unknown. In the present study, MeJA treatment not only alleviated CI but also promoted polyamines accumulation during most of the storage periods (Table 1). Treatment with nor-NOHA reduced the chilling tolerance induced by MeJA treatment but had little effect on MeJA-induced polyamines contents. These results indicate that other factors aside from polyamines are required for MeJA-induced chilling tolerance in tomato fruit. The potential role of polyamines in the chilling tolerance induced by MeJA must be elucidated in the future studies.

In higher plants, putrescine is synthesized by two routes. The distinct ornithine decarboxylase-catalyzed and arginine decarboxylase (ADC)-catalyzed pathways have been thoroughly characterized (Groppa and Benavides, 2008). However, the effects of changes in arginase activity on putrescine levels have not yet been reported. In the present investigation, the increased putrescine content in MeJA-treated fruit remained almost unchanged by inhibiting arginase activity with nor-NOHA pretreatment. Our results suggested that arginase inhibition probably increased the availability of arginine for ADC. Alternatively, arginase may not be essential for MeJA-induced putrescine accumulation in tomato fruit. However, further research is necessary to make insights into the roles of arginase in MeJA-induced synthesis of putrescine.

Conclusions

The present study showed for the first time that arginase may be involved in the chilling tolerance induced by MeJA treatment in tomato fruit, possibly by a mechanism ameliorating antioxidant enzyme activities and increasing proline levels. However, whether and how arginase activities will alter the synthesis of polyamines in tomato fruit during cold storage should be future investigated.

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