Postharvest Biology and Technology 64 (2012) 160–167

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Methyl jasmonate alters arginine catabolism and improves postharvest chilling tolerance in cherry tomato fruit

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ARTICLE INFO

Article history:
Received 21 April 2011
Accepted 18 July 2011

Keywords:
Arginine catabolism
Arginine-related amino acids
Chilling injury
Methyl jasmonate
Polyamine
Tomato fruit

ABSTRACT

The influence of methyl jasmonate (MeJA) on levels of gene transcripts, enzyme activities and metabolites related to arginine catabolism and chilling injury (CI) was studied in cherry tomato (*Solanum lycopersicum* L. cv. Messina) fruit stored at 2 °C for 21 days. The CI index of fruit pretreated with 0.05 mM MeJA vapor for 12 h at 20 °C was reduced compared with that of untreated fruit. The reduction was associated with up-regulated arginine catabolism. The mRNA levels and activities of arginase, arginine decarboxylase (ADC) and ornithine aminotransferase (OAT) in treated fruit were higher than in control fruit, while those of ornithine decarboxylase (ODC) increased transiently in response to MeJA treatment during the early days of cold storage. Free putrescine (Put) and proline accumulated in MeJA-treated fruit, while levels of other arginine-related amino acids were affected by MeJA. Spermidine (Spd) and spermine (Spm) contents remained unchanged in response to MeJA treatment over most of the storage time. The results indicate that MeJA becomes involved in coordinated catabolism of arginine, and helps to improve chilling tolerance in cherry tomato fruit.

1. Introduction

Arginine, a basic amino acid with high nitrogen to carbon ratio, is one of the most functionally versatile amino acids in living cells. Arginine has been found to be 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) (Wu and Morris, 1998; Jubault et al., 2008; Gao et al., 2009). There has been widespread interest in arginine because it is involved in multiple metabolic processes in animal (Morris, 2007, 2009) and plant (Goldraij and Polacco, 2000; Jubault et al., 2008) systems. Nitric oxide synthase (NOS), arginase and arginine decarboxylase (ADC) are key enzymes in arginine catabolism. In mammalian cells, arginine can be catabolized in a tissue-specific manner by NOS, which metabolizes arginine to produce NO and citrulline (Wu and Morris, 1998). However, the genes encoding NOS proteins and animal-type NOS in higher plants remain elusive (Guo et al., 2003; Zemojtel et al., 2006). The second arginine catabolic pathway is catalyzed by arginase, which hydrolyzes arginine to ornithine and urea. Ornithine contributes to the biosynthesis of polyamines and proline through ornithine decarboxylase (ODC) and ornithine aminotransferase (OAT), respectively (Hervieu et al., 1995; Alabadí et al., 1996). In higher plants, polyamines are also synthesized by the decarboxylation of arginine catalyzed by ADC (Alabadí et al., 1996). Moreover, it has been widely reported that polyamines accumulation in plants is generally due to the increase in ADC activity in response to abiotic stress (Hao et al., 2005; Groppa and Benavides, 2008). Therefore, the different pathways for arginine catabolism may be associated with various physiological processes and stress responses.

One of the main postharvest problems affecting tropical and subtropical commodities is their sensitivity to low temperature, resulting in chilling injury (CI). This limits storage life and leads to significant degradation of produce quality. Methyl jasmonate (MeJA), a natural plant growth regulator, plays important roles in plant growth and development, fruit ripening, and responses to environmental stress (Imanishi et al., 1998; Walia et al., 2007; Ziosi et al., 2009). Treatment with MeJA can reduce the development of CI symptoms in a number of horticultural crops, including loquat, tomato and peach fruit (Ding et al., 2002; Meng et al., 2009; Cao et al., 2010). Thus, MeJA has a potential application in postharvest treatments for alleviating CI and maintaining quality. However, the mode of action of MeJA in reducing CI and quality deterioration has not been clearly elucidated. It has recently been shown that altered levels of arginine-related metabolites (free polyamines or proline), and changes in some enzymatic (arginase, ADC and/or ODC) activities and/or their gene transcript levels involved in arginine catabolism occur in response to MeJA treatment (Biondi et al., 2001; Chen et al., 2006; Haggag and Abd-El-Kareem, 2009). Some studies have shown that both polyamines and proline accumulate

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doi:10.1016/j.postharvbio.2011.07.006

in plants in response to chilling stress. An increase in putrescine (Put) or proline levels is correlated with improved cold tolerance in chilling-sensitive plants (Akiyama and Jin, 2007; Yadegari et al., 2007; Zhang et al., 2009). It is known that arginine is one of the precursors for biosynthesis of polyamines and proline. Moreover, treatment with arginine can alleviate the CI of cherry tomato fruit during cold storage (Zhang et al., 2010a,b). Consequently, arginine catabolism is potentially involved in chilling tolerance of plants.

Cherry tomato fruit, a typical chilling sensitive horticultural crop, is an excellent model system in which to investigate the physiological and molecular mechanisms of postharvest CI in fruit during cold storage. The present study was conducted to investigate whether the MeJA-induced chilling tolerance in cherry tomato fruit is linked to changes in arginine catabolism.

2. Materials and methods

2.1. Plant material and treatments

Cherry tomato (*Solanum lycopersicum* L. cv. Messina) fruit at the mature green stage were used as materials. Blemish-free fruit of uniform size and shape were selected and randomly divided into two lots. Each lot containing three replicates of 180 fruit that were treated with either 0 (control) or 0.05 mM MeJA (Sigma, St. Louis, USA) vapor in an air-tight container for 12 h in darkness at 20 °C. After treatment the containers were opened, and the fruit were stored at 2 ± 1 °C with a relative humidity of 80–90% for up to 21 days.

Untreated and treated fruit were sampled on day 0, and after 1, 3, 7, 14 and 21 days of cold storage. Equatorial slices from sampled fruit were diced, frozen in liquid nitrogen and stored at −80 °C for gene, enzyme, polyamines and amino acid analysis. For CI evaluation, fruit of each treatment were sampled weekly from cold storage and held at 20 °C for 3 days.

2.2. Chilling injury evaluation

Symptoms of CI, manifested as surface pitting were assessed according to the method of Ding et al. (2002), where symptoms was assessed visually: 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 (%) = $\sum$ [(CI level) × (number of fruit at the CI level)]/[(total number of fruit) × 4] × 100.

2.3. Quantitative real-time PCR (qPCR)

Total RNA was extracted from approximately 2 g of frozen tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA were obtained as described by Zhao et al. (2009a,b). Transcript levels of *LeARG1* (AY656837), *LeARG2* (AY656838), *ADC* (L16582), *ODC* (AF029349) and *OAT* (AY897573) were evaluated via qPCR using the SYBR Green Master mix kit (Toyobo, Osaka, Japan) on a Chromo4 real time PCR Detection System (Bio-Rad, Hercules, USA). For each sample, three replicates were performed in a final volume of 25 μL containing 20 ng cDNA, 0.4 μM of specific primers and 12.5 μL SYBR Green MasterMix according to the manufacturer's instructions. The reference gene was ubiquitin (*Ubi3*), which was proven to be stable under several conditions and frequently used as a single gene to normalize the quantification of expression in tomato (Zhao et al., 2009a,b; Zhang et al., 2010a,b) Specific primers of each gene were as reported in Table 1. The qPCR 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. To determine relative fold differences for each sample, the threshold constant (Ct) value was normalized to the Ct value for *Ubi3*, and set relative to control samples (0 day) according to the formula $2^{-\Delta\Delta Ct}$.

2.4. Arginase assays

Frozen tissues (2 g) were ground in a mortar with liquid nitrogen and homogenized in 6 mL of 100 mM Tris–HCl (pH 7.5) containing 1% (v/v) 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride and 0.5% (w/v) polyvinyl polypyrrolidone (PVPP). Homogenates were centrifuged at 12,000 × *g* for 15 min at 4 °C, and the supernatants were used as the enzyme source. Arginase activity was measured using a spectrophotometric assay for detection of urea as described previously (Chen et al., 2004), with minor modifications. The enzyme solution was activated with 1 mM $MnCl_2$ at 37 °C for 15 min. Reaction mixtures contained 20 μL of the supernatant in 100 mM Gly-NaOH (pH 9.6), 100 mM arginine (pH 9.6) and 2 mM $MnCl_2$ in a total volume of 0.5 mL. Reactions were carried out at 37 °C for 20 min and stopped by adding 0.5 mL of 15% (v/v) perchloric acid ($HClO_4$). An aliquot of 250 μL was mixed vigorously with 3 mL of a mixture of sulfuric (27% (v/v)) and phosphoric acid (9% (v/v)) and 100 μL of 3% (w/v) α-isonitrosopropiophenone in 95% (v/v) ethanol. The mixtures were boiled in the dark for 60 min and cooled for 10 min to room temperature. The absorbance was recorded at 520 nm and urea standards were prepared for calibration. Arginase activity was expressed as nmol urea produced per min per mg of protein.

2.5. ADC and ODC assays

The enzymes, ADC and ODC were extracted as described by Alabadí et al. (1996). Frozen tissues were ground in a mortar with liquid nitrogen and homogenized in 5 volumes

Table 1
Genes and oligonucleotides used in the qPCR experiments.

Genes	Loci	Encoded proteins	Primers (5′-3′)
LeARG1	AY656837	Arginase	GTGGAAAAAGGACAGAATCG
			AGAGACGTTGAGGCTACAGC
LeARG2	AY656838	Arginase	TGTTCGTGGACTTGGAGGTG
			CCCTGGAGAAATGAAGAGTTGTG
ADC	L16582	Arginine decarboxylase	GTGATCGTAAGGGCGGAAAG
			GCACGGGCATCTTCATTGAG
ODC	AF029349	Ornithine decarboxylase	AAACCCACTTCCACGACTTCC
			GACTCTTTTGCCGATGATGGTT
OAT	AY897573	Ornithine aminotransferase	CAGAACAGGCTCAAATGCTCAC
			CATATAACCCCATTTCCTTGCC
Ubi3	X58253	Ubiquitin	TCCATCTCGTGCTCCGTCT
			CTGAACCTTTCCAGTGTCATCAA