Scientia Horticulturae 179 (2014) 349–355

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Metabolism of endogenous arginine in tomato fruit harvested at different ripening stages

Xinhua Zhang, Nana Ji, Fanghui Zhen, Peipei Ren, Fujun Li*

School of Agricultural and Food Engineering, Shandong University of Technology, Zibo 255049, Shandong, PR China

ARTICLE INFO

Article history:
Received 11 August 2014
Received in revised form
19 September 2014
Accepted 24 September 2014
Available online 22 October 2014

Keywords:
Arginine metabolism
Arginine-related amino acids
Polyamines
Ripening
Tomato fruit

ABSTRACT

Tomato (*Solanum lycopersicum* cv. Messina) fruit, at five ripening stages (mature green, breaker, pink, light red and full red), have been analyzed for the expression of genes involved in arginine metabolism, polyamines and arginine-related amino acids content, as well as the nitric oxide synthase (NOS) activity and nitric oxide (NO) content. During ripening, the expression of *LeARG1* and *LeARG2*, two genes encoding arginase, and NOS activity, as well as NO content decreased and the highest levels of them were found in mature green fruit. The expression of gene encoding arginine decarboxylase (ADC) increased with fruit ripening and reached the highest value at pink stage, which possibly contributed to the increased polyamines concentration. While the ornithine decarboxylase (ODC) might play a minor role in polyamines biosynthesis during tomato fruit ripening, the gene expression pattern of which differed with that of polyamines accumulation. The expression of gene encoding ornithine aminotransferase (OAT) increased during the later ripening stages of tomato, which was accompanied by proline accumulation. From all the amino acids tested, glutamate content was the most abundant and showed a marked increase during the course of fruit ripening. In contrast, arginine and ornithine contents remain relatively uniform throughout fruit ripening. These results implicate that the unique physiological roles of arginine in fruit ripening may depend on the coordination of different pathways of arginine metabolism.

1. Introduction

Arginine, a basic amino acid with a high N/C ratio (four nitrogen and six carbon atom per molecule), serves as an important nitrogen reserve in many plants (Micallef and Shelp, 1989; Gao et al., 2009). Additionally, the physiological importance of arginine for promoting and maintaining cell function is now widely accepted, stemming from the recognition that arginine is one of the most versatile amino acids. Not only is it the major source of the nitrogen atom in the biosynthesis of nitric oxide (NO), but it can positively modulate several subsequent ornithine-dependent pathways, including proline-, glutamate- and ornithine-dependent polyamines biosynthesis (Corpas et al., 2006; Morris, 2007; Jubault et al., 2008). It is therefore no surprise that there is diversity of enzymes involved in the metabolism of arginine. Arginine decarboxylase (ADC), arginase, and nitric oxide synthase (NOS) are the key enzymes in arginine metabolism (Fig. 1). ADC catalyzes arginine to produce polyamines, whereas arginase hydrolyzes arginine to urea and ornithine, the latter of which is a precursor for polyamines and proline biosynthesis via ornithine decarboxylase (ODC) and ornithine aminotransferase (OAT), respectively. In mammalian cells, most of the NO produced is due to NOS, which catalyzes arginine to produce NO and citrulline (Morris, 2007). Although the genes encoding NOS proteins and animal-type NOS in higher plants remain elusive, an arginine-dependent NO synthesis, similar to that in animals, has also recently been described in plants (Corpas et al., 2006; Guo et al., 2003).

Arginine can account for 50% of the nitrogen in seed protein, and up to 90% of the free nitrogen in vegetative tissues. To date, arginine metabolism via arginase as a source of nitrogen for the biosynthesis of nitrogenous compounds during seeds germination and post-germinative growth has been intensely investigated (Goldraij and Polacco, 2000; Todd et al., 2001). Compared with the early seedling growth, fruit ripening can be considered as a feature of plant organ senescence in which a coordinated series of physiological and biochemical changes take place and the demand for nitrogen may vary significantly.

The metabolites of arginine, especially NO, polyamines and proline, are all multifunctional molecules in plant, mediating an array of physiological and biological processes, including responses to abiotic and biotic stresses, programmed cell death, stomatal closure, seed generation, and root development (Arasimowicz and

* Corresponding author. Tel.: +86 533 2786820; fax: +86 533 2786820.
E-mail addresses: lifujun@sdut.edu.cn, lifujun2020@163.com (F. Li).

http://dx.doi.org/10.1016/j.scienta.2014.09.045
0304-4238/

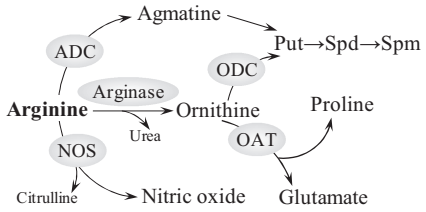

Fig. 1. Overview of arginine metabolism pathways in tomato. Only enzymes that directly use arginine or ornithine are indicated. For the sake of clarity, not all reactants and products are shown. ADC, arginine decarboxylase; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; OAT, ornithine aminotransferase; Put, Putrescine; Spd, Spermidine; Spm, Spermine.

Wieczorek, 2007; Groppa and Benavides, 2008; Delauney and Verma, 1993). Furthermore, recent evidence indicated that NO and polyamines in their free forms may have anti-ripening and senescence properties. Application of exogenous NO or polyamines could extend the postharvest life of some fresh horticultural produces (Eum et al., 2009; Manjunatha et al., 2010; Valero et al., 2002). While most of the research concerning proline metabolism in plants has been focused on its accumulation in vegetative tissues in response to abiotic stresses such as drought and salinity (Delauney and Verma, 1993). Stress-induced accumulation occurs predominantly through the enhanced biosynthesis of proline from glutamate via the pathway catalyzed by Δ^1-pyrroline-5-carboxylate synthase rather than from ornithine via the pathway catalyzed by OAT (Delauney and Verma, 1993). The involvement of proline in grapevine fruit ripening have also been reported, which indicated that proline accumulation during normal plant development are independent of those operating in response to abiotic stress, but the data available are still scarce (Stines et al., 1999).

Despite the fact that NO and proline can also be generated by activation of pathways or processes independent of arginine metabolism, arginine as the major source for the biosynthesis of NO and proline, as well as polyamines have been proved in plant. Therefore, analysis of the switch between the different branches of arginine metabolism and the homeostasis of these metabolites will be useful to gain further insights into the diverse roles of arginine in the ripening process of fruits and vegetables.

Tomato is a good model system for studying the ripening process, the ripening stages of which can be easily defined based on the fruit color changes from green to red. In the present work, the different pathways of arginine metabolism at the transcriptional, enzymatic, and metabolic levels in tomato fruit of different ripening stages were investigated, aiming at gaining a better understanding of the physiological roles of arginine in the ripening process of fruits and vegetables.

2. Materials and methods

2.1. Plant materials

Tomato (*Solanum lycopersicum* cv. Messina) plants were grown in a local greenhouse under standard culture conditions (27/19 °C; 16-h light/8-h dark; relative humidity 70%). Tomato fruit at mature green (I), breaker (II), pink (III), light red (IV) and full red (IV) stages were separately harvested. The ripening stages of tomato fruits were defined as described earlier (Boller et al., 1979). On this scale, "mature green fruits" have reached full size but are entirely green. "Breaker" fruits show no more than 10% coloration and "pink" fruits 30–60% coloration. "Light-red" fruits are more than 60% pink but less than 90% red while "full red" fruits are more than 90% red. Twenty fruits of uniform size and free from defects and blemishes were selected at each ripening stages (one replication). There were three replications of each ripening stages in total. The mesocarp from the fruit equator area was cut into small pieces, quickly frozen in liquid nitrogen, and stored at −80 °C until further analysis.

2.2. RNA extraction and quantitative real-time PCR (qPCR) assay

Total RNA were extracted from frozen fruit samples using trizol method as described previously (Zhang et al., 2010). Any remaining genomic DNA was removed by digestion with RNase-free DNase I (Promega, Madison, WI, USA) for 45 min at 37 °C. The cDNA was synthesized using oligo(dT)15 primer and 2 μg of total RNA treated with RNase-free DNase I and M-MLV Reverse Transcriptase (Promega). The synthesized cDNA was used for subsequent qPCR analysis 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 the Primer Express software (Applied Biosystems) and their nucleotide sequences are listed in Table 1. The Ubi3 encoding ubiquitin was used as the reference gene. 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. All experiments were run in triplicate with different cDNAs synthesized from three biological replicates. The abundance of targeted gene transcripts was normalized to *Ubi3* mRNA and set relative to mature green samples according to the $2^{-\Delta\Delta Ct}$ method.

2.3. Free polyamine determination

Polyamines analyses were performed according to the method of Flores and Galston (1982) with slight modifications. Fruit samples were homogenized in four volumes of 5% (w/v) cold perchloric acid and centrifuged at 20,000 × *g* for 30 min at 4 °C. Aliquots (2 mL)

Table 1
Genes and oligonucleotides used in the quantitative real-time PCR.

Genes	Loci	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
OAT	AY897573	Ornithine aminotransferase	Forward: CAGAACAGGCTCAAATGCTCAC Reverse: CATATAACCCCATTTCCTTGCC
Ubi3	X58253	Ubiquitin	Forward: TCCATCTCGTGCTCCGTCT Reverse: CTGAACCTTTCCAGTGTCATCAA