Plant Physiology and Biochemistry 100 (2016) 27–36

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Spatial and temporal distribution of genes involved in polyamine metabolism during tomato fruit development

Georgios Tsaniklidis [a], Anastasios Kotsiras [b], Athanasios Tsafouros [a], Peter A. Roussos [a], Georgios Aivalakis [a], Panagiotis Katinakis [a], Costas Delis [b, *]

[a] Agricultural University of Athens, Department of Natural Resources Development and Agricultural Engineering, Iera Odos 75, 11855 Votanikos, Athens, Greece

[b] Technological Educational Institute of Peloponnese, School of Agricultural Technology and Food Technology and Nutrition, Department of Agricultural Technology, 24100 Antikalamos, Kalamata, Greece

ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form
4 January 2016
Accepted 4 January 2016
Available online 5 January 2016

Keywords:
Fruit development
Polyamine biosynthesis
Polyamine catabolism
Arginine decarboxylase
Ornithine decarboxylase
Copper containing amine oxidase
Expression analysis

ABSTRACT

Polyamines are organic compounds involved in various biological roles in plants, including cell growth and organ development. In the present study, the expression profile, the accumulation of free polyamines and the transcript localisation of the genes involved in Put metabolism, such as Ornithine decarboxylase (*ODC*), Arginine decarboxylase (*ADC*) and copper containing Amine oxidase (*CuAO*), were examined during *Solanum lycopersicum* cv. Chiou fruit development and maturation. Moreover, the expression of genes coding for enzymes involved in higher polyamine metabolism, including Spermidine synthase (*SPDS*), Spermine synthase (*SPMS*), S-adenosylmethionine decarboxylase (*SAMDC*) and Polyamine oxidase (*PAO*), were studied. Most genes participating in PAs biosynthesis and metabolism exhibited an increased accumulation of transcripts at the early stages of fruit development. In contrast, *CuAO* and *SPMS* were mostly expressed later, during the development stages of the fruits where a massive increase in fruit volume occurs, while the *SPDS1* gene exhibited a rather constant expression with a peak at the red ripe stage. Although Put, Spd and Spm were all exhibited decreasing levels in developing immature fruits, Put levels maxed late during fruit ripening. In contrast to Put both Spd and Spm levels continue to decrease gradually until full ripening. It is worth noticing that *in situ* RNA–RNA hybridisation is reported for the first time in tomato fruits. The localisation of *ADC2*, *ODC1* and *CuAO* gene transcripts at tissues such as the locular parenchyma and the vascular bundles fruits, supports the theory that all genes involved in Put biosynthesis and catabolism are mostly expressed in fast growing tissues. The relatively high expression levels of *CuAO* at the ImG4 stage of fruit development (fruits with a diameter of 3 cm), mature green and breaker stages could possibly be attributed to the implication of polyamines in physiological processes taking place during fruit ripening.

1. Introduction

Polyamines (PAs), such as the diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm), are low molecular weight aliphatic cations that are ubiquitous in almost all living organisms (Hussain et al., 2011). In plants, PAs are implicated in many developmental processes, such as cell division and differentiation, root growth and development, flower development, stress tolerance, senescence processes, DNA synthesis, gene transcription, cell wall loosening and fruit maturation (Delis et al., 2005; Gill and Tuteja, 2010; Shi and Chan,

Abbreviations: Put, Putrescine; Spd, Spermidine; Spm, Spermine; SAM, S-adenosylmethionine; ODC, Ornithine Decarboxylase; ADC, Arginine Decarboxylase; CuAO, Copper containing Amine Oxidase; SPDS, Spermidine Synthase; SPMS, Spermine Synthase; SAMDC, S-adenosylmethionine Decarboxylase; PAO, Polyamine Oxidase; PA, Polyamines; AOs, amine oxidases; ImG, Imature Green; MG, Mature Green; Br, Breaker; RR, Red Ripe; DMFO, Difluoromethylornithine.

* Corresponding author. Postal address: Antikalamos, 24100, Kalamata, Greece.
E-mail addresses: giorgos.tsaniklidis@gmail.com (G. Tsaniklidis), akotsiras@teikal.gr (A. Kotsiras), thantsaf@hotmail.com (A. Tsafouros), roussosp@aua.gr (P.A. Roussos), gaivalakis@aua.gr (G. Aivalakis), katp@aua.gr (P. Katinakis), delis@teikal.gr (C. Delis).

http://dx.doi.org/10.1016/j.plaphy.2016.01.001
0981-9428/

2014). Although in mammals Put is exclusively synthesised directly from ornithine via ornithine decarboxylase (ODC; EC 4.1.1.7), in plant cells Put is alternatively biosynthesised indirectly by arginine through arginine decarboxylase (ADC; EC 4.1.1.19) (Agudelo-Romero et al., 2014, 2013). The biosynthesis of higher PAs, Spd and Spm is mediated by Spermidine synthase (SPDS; EC 2.5.1.16), and Spermine synthase (SPMS; EC 2.5.1.22) respectively, while S-adenosylmethionine decarboxylase (SAMDC; 4.1.1.50) converts S-adenosylmethionine (SAM) to decarboxylated S-adenosylmethionine which adds amino propyl groups to Put in a sequential manner for the synthesis of Spd and Spm (Bhatnagar et al., 2001; Neily et al., 2011). SAM however, also serves as an ethylene biosynthesis precursor suggesting a critical balance between PA and ethylene biosynthesis (Van De Poel et al., 2013).

The inter-conversion cycle of PAs is tightly regulated by Polyamine oxidase (PAO; EC 1.5.3.11) and copper-containing amine oxidase (CuAO; EC 1.4.3.6) (Moller and McPherson, 1998; Moschou et al., 2012). It is well established that both enzymes involved in PA catabolism (PAO and CuAO) are mainly located in cell walls, while both enzyme activities release H_2O_2 as a byproduct of PA catabolism (Angelini et al., 1993; Sebela et al., 2001; Cona et al., 2003). Spm and Spd catabolism and derived apoplastic H_2O_2 have been implicated in abiotic stress response signaling and lignin production in apoplastic space adjoining plasma membrane (Moschou et al., 2008). Furthermore, CuAO derived H_2O_2 is correlated to tissue development and cell expansion in soybean seedlings (Delis et al., 2006).

Tomato fruit development and ripening are considered to be complex processes, in which various physiological and biochemical modifications take place including cell expansion, tissue development and changes in colour, organic acids, nutrients and sugars that finally lead to the development of its' distinct organoleptic characteristics (Fujisawa et al., 2011). In most fruits, PAs were found to be abundant in developing fruits, where cell division and cell expansion mostly take place (Yahoo et al., 2001; Liu et al., 2006). Although PAs are usually found at relatively low concentrations in mature fruits, they are considered to be actively involved especially in climacteric fruit ripening, primarily regulating the ethylene emission by antagonism (Bregoli et al., 2002; Liu et al., 2006). The exact mechanism through which PAs affect ripening is mostly unknown (Agudelo-Romero et al., 2013). The interrelationship between PAs and ethylene biosynthesis due to antagonism for S-adenosylmethionine (SAM), which comprises a common precursor compound, is well established (Srivastava et al., 2007; Agudelo-Romero et al., 2013). It has also been suggested that enzymes producing H_2O_2 like amine oxidases (including CuAO and PAO) along with their contribution to PA homeostasis, are implicated to senescence processes leading to fruit maturation (Delis et al., 2006; Mateos et al., 2013).

In the present study, *in silico* analysis in tomato genomic databases revealed the presence of ten genes primarily involved in PA metabolism. Transcript accumulation of *ODC1,2, ADC1,2*, *SPDS1,2*, *SPMS*, *SAMDC1,2*, *PAO* and *CuAO* and the concentration of free Put, Spd, Spm and total free PAs were examined during tomato fruits development and ripening. In addition, the spatial contribution of *ODC1,2*, *ADC2* and *CuAO* genes transcripts was studied in developing tomato fruits by *in situ* RNA–RNA hybridisation, in order to decipher PA transcriptional regulations and investigate the possible correlation of PA's metabolism to physiological processes, such as fruit ripening and development. It is worth noticing that for the first time an *in situ* hybridization approach has been used for the spatial distribution of gene transcripts in tomato fruits. Moreover, a unique gene coding for a putative CuAO in tomato fruits has been sequenced and characterized.

2. Materials and methods

2.1. Plant material and growth conditions

Plants of cherry tomato (*Solanum lycopersicum* cv. Chiou; a Greek traditional variety with high organoleptic qualities and long shelf life), were cultivated in a glasshouse of the Technological Institute of Kalamata, Greece between February and May. The mean minimum and maximum temperatures in the greenhouse were 16.3 ± 2.0 °C and 27.4 ± 4 °C accordingly, (Spring, {March–May}) and 13.2 ± 2 °C and 24.2 ± 4 °C (Winter, {Oct–Feb}). Average solar radiation was 15.5 MJ/m^2 per day. Whole fruits were harvested systematically at the following stages: 0.5 cm in diameter (ImG1, 7 DAF {days after flowering}) 1 cm in diameter (ImG2-14 DAF), 2 cm in diameter (ImG3-21 DAF), 3 cm in diameter (ImG4-28 DAF), Mature Green (MG-42 DAF), change of color-Breaker (Br-51 DAF), red ripe (RR-60 DAF). Fruits of ImG2 and ImG4 were used for *in situ* RNA–RNA hybridisation, while fruits of all stages of fruit development were used for the study of transcript accumulation and free PAs detection.

Each harvest was carried out at 11.00 AM and replicated three times. Samples were immediately frozen in liquid nitrogen, homogenised using a pestle and mortar and then stored at −80 °C.

2.2. Characterization of a cDNA clone coding for CuAO

By performing BLAST searches among the genomic tomato (http://solgenomics.net) and in the National Center for Biotechnology Information databases (http://www.ncbi.nlm.nih.gov), a single genetic locus coding for the *CuAO* gene has been detected in the tomato genome and a cDNA clone coding for *CuAO* was identified and obtained from ResGen, Invitrogen Corp. Each nucleotide sequence was determined and since 5′ end was missing, total RNA was extracted from fruit at 3 cm stage of fruit development (ImG4) and a 5′ RACE-PCR was performed with the SMART 5′ RACE kit from Clontech (Westburg, NL). The amplified fragment was cloned into the pBlueScriptKS + plasmid vector (Stratagene) and fully sequenced. The full-length cDNA clone was designated as CuAO. The Clustal algorithm was used for analysis and determination of the relatedness of the deduced polypeptides to related sequences from other species. Significant similarities between AJ871578 from tomato cv Chiou and CuAO form *Arabidopsis thaliana* were detected. Tomato CuAO exhibited 45% similarity to *A. thaliana* ATAO1 (AT4G14940) (Møller and McPherson, 1998) and 59% similarity to AtCuAO1 (AT1G31670) CuAOs respectively. Moreover, important for the enzymatic activity amino acid residues that are conserved among already characterized plant CuAOs were also present in tomato CuAO amino acid sequence (Supplementary data).

2.3. Study of transcript accumulation-qPCR

Quantitative PCR (qPCR) experiments were conducted as previously described (Delis et al., 2011). Total RNA was isolated from three replicates of each sample using the RNeasy extraction Kit (Qiagen). Total RNA was quantified spectrophotometrically by measuring the absorbance at 260 nm and the absorbance ratio of 260/280 nm in Nanodop (Thermo) and on 3% w/v agarose gel. In order to eliminate genomic DNA, samples were treated with DNase I (Takara) at 37 °C for 60 min. Ubiquitin (*UBQ*) primers were used on qPCR to test for complete DNA removal using *S. lycopersicum* genomic DNA as the positive control.

First strand cDNA was reverse transcribed with Affinity ScriptTM Multi Temperature (Stratagene) from 2 lg of DNase-treated total RNA at 42 °C for 5 min and 55 °C for 60 min using oligo(dT) primer according to the manufacturer's instructions. The