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Research paper

Genome-wide identification, phylogenetic analysis, and expression profiling of polyamine synthesis gene family members in tomato

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ABSTRACT

Polyamines (PAs), including putrescine (Put), spermidine (Spd), spermine (Spm), and thermospermine (T-Spm), play key roles in plant development, including fruit setting and ripening, morphogenesis, and abiotic/biotic stress. Their functions appear to be intimately related to their synthesis, which occurs via arginine/ornithine decarboxylase (ADC/ODC), Spd synthase (SPDS), Spm synthase (SPMS), and Acaulis5 (ACL5), respectively. Unfortunately, the expression and function of these PA synthesis-relate genes during specific developmental process or under stress have not been fully elucidated. Here, we present the results of a genome-wide analysis of the PA synthesis genes (*ADC*, *ODC*, *SPDS*, *SPMS*, *ACL5*) in the tomato (*Solanum lycopersicum*). In total, 14 PA synthesis-related genes were identified. Further analysis of their structures, conserved domains, phylogenetic trees, predicted subcellular localization, and promoter cis-regulatory elements were analyzed. Furthermore, we also performed experiments to evaluate their tissue expression patterns and under hormone and various stress treatments. To our knowledge, this is the first study to elucidate the mechanisms underlying PA function in this variety of tomato. Taken together, these data provide valuable information for future functional characterization of specific genes in the PA synthesis pathway in this and other plant species. Although additional research is required, the insight gained by this and similar studies can be used to improve our understanding of PA metabolism ultimately leading to more effective and consistent plant cultivation.

1. Introduction

Polyamines (PAs) are small aliphatic amines that are found in both prokaryotic and eukaryotic organisms (Kusano et al., 2008). In plants, PAs play key roles in development and morphogenesis, including senescence, fruit set, and ripening (Bregoli et al., 2002; Metabolism et al., 2018; Valero et al., 1998), as well as their response to abiotic and biotic stresses (Ebeed et al., 2017). The most abundant PAs in plants are putrescine (Put), spermidine (Spd), and spermine (Spm), while cadaverine, thermospermine, norspermidine, and norspermine are less abundant (Metabolism et al., 2018). PA content and function are largely regulated by changes in their synthesis and breakdown.

Synthesis of Put proceeds through arginine decarboxylase (ADC) via arginine or/and ornithine decarboxylase (ODC) from ornithine, respectively, while conversion of Put to Spd requires Spd synthase (SPDS). The biosynthesis of Spm and T-Spm are mediated by spermine synthase (SPMS) and (ACL5), respectively. The amino propyl groups used for the synthesis of Spd and Spm are generated by S-adenosylmethionine decarboxylase (SAMDC) during the conversion of S-adenosylmethionine (SAM) to decarboxylated S-adenosylmethionine. Interestingly, SAM is a precursor in the synthesis of ethylene, suggesting a critical balance between PAs and ethylene biosynthesis (Hussain et al., 2011; Kusano et al., 2008). Put catabolism is primarily mediated by diamine oxidases (CuAOs), while the terminal catabolism

Abbreviations: ADC, arginine decarboxylase; ODC, ornithine decarboxylase; PA, polyamines; Put, putrescine; Spd, spermidine; Spm, spermine; T-Spm, thermospermine; SPDS, Spd synthase; SPMS, Spm synthase; ACL5, Acaulis5

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of triamine Spd and tetramine Spm is modulated by PA oxidases (PAOs) (Cona et al., 2006; Kim et al., 2014; Kusano et al., 2008; Liu et al., 2014a, 2014b; Planas-Portell et al., 2013).

Several studies have suggested a key role for PAs during fruit set, an important process in flowering plants (Alabadí et al., 1995; Carbonell and Navarro, 1989). Indeed, early studies in pea plants have shown that Spm levels are decreased in self-pollinated ovaries, but increase at the onset of senescence in the unpollinated ovaries (Gomez-Jimenez et al., 2010). PA function during fruit set has also been reported in apples (Biasi et al., 1988), grapes (Shiozaki et al., 2000), mangoes (Malik and Singh, 2004), oranges (Tassoni et al., 2004), peaches (Liu et al., 2006; Ziosi et al., 2006), and tomatoes (Rastogi and Davies, 1991). In general, higher PA content has been observed in the early stages of fruit development accompanied by a gradual decrease during fruit ripening. In avocado, for example, a PA burst occurs at the onset of fruit development, then declined until the ripe stage (Kushad et al., 1988). Notably, differences in PA content have also been noted between climacteric and non-climacteric fruit. In non-climacteric grapes, Put and Spd strongly decrease during ripening, whereas Spm had more or less constant levels during ripening (Shiozaki et al., 2000). In climacteric tomatoes, Put levels increase progressively during fruit maturation, reaching its maximum at the red ripe stage, while Spd and Spm levels were at their lowest at this stage (Tsaniklidis et al., 2016). Thus, PA content and the functions of various PAs appear to differ depending on the fruit as well as the ripening stage and fruit set. These differences appear to be largely related to the enzymes involved in PA metabolism and synthesis. In fact, during ovary enlargement in tobacco plants, ODC activity was significantly increased between the anthesis and fertilization stages, while content of PAs remained constant until fertilization when they increased dramatically (Slocum and Galston, 1985).

PAs are also implicated in the plant response to abiotic and biotic stresses, including decreased water supply, temperature changes, and altered salt concentrations, whereby incremental changes in PA concentration are controlled by fine-tuned regulation of the enzymes involved in the PA biosynthesis and catabolism (Alcázar et al., 2010; Hussain et al., 2011; Kusano et al., 2008; Liu et al., 2011; Minocha et al., 2014; Pál et al., 2015; Shi and Chan, 2014; Tiburcio et al., 2014). In rice, for example, the expression of ADC and ODC were correlated to drought tolerance (Do et al., 2013). In tomatoes, DAO and PAO activity are increased to mediate cold tolerance via changes in Put levels, and inhibition of ADC was shown to decrease cold tolerance (Song et al., 2015). Changes in enzymatic activity and subsequent PA levels were also observed in response to temperature decreases in cold-tolerant rice cultivar (Do et al., 2013) and cucumber plants (Shen et al., 2000). Furthermore, in *Arabidopsis*, the deletion of SPMS genes results in higher tolerance to salt conditions (Alet et al., 2011), while overexpression of SPMDS1 in the European pear caused Spd accumulation and improved tolerance to osmotic stress (He et al., 2008). PAs also act as antioxidants, stabilizers of nucleic acids and biomembranes, and regulators of cytosolic pH to protect against abiotic factors (Romero et al., 2018). While there are an extensive number of published studies investigating these processes, the full mechanism by which PAs participate in various plant processes remains largely unknown.

In the present study, we performed an in-silico analysis using a tomato genomic database to investigate the expression of PA-related genes during stress. In doing so, we have identified 14 candidate genes involved in PA synthesis. Further analysis of their gene structures, phylogenetic relationships, organ specific expression profiles, and expression patterns under various hormones and stress treatments have provided insight into various plant processes, including fruit set and ripening as well as the biological responses to both stress (e.g., heat, injury, drought, etc.) and developmental hormones. To our knowledge, this is the first time this type of analysis has been performed to elucidate the mechanisms underlying PA function in tomatoes. Thus, these data provide a solid basis for further functional characterization of specific genes in the PA synthesis pathway in this and other plant species.

2. Materials and methods

2.1. Sequence retrieval

As the majority of *Arabidopsis* genes involved in PA biosynthesis have been reported previously, all of their protein sequences were extracted from the *Arabidopsis* Information Resource (TAIR) database (http://www.arabidopsis.org) and used as queries for BLASTP analysis with an e-value threshold of $< 1e^{-0}$ in the Sol Genomic Network database (SGN; https://www.sgn.cornell.edu). Various PA synthesis gene sequences were also retrieved for other species from the NCBI database.

2.2. Phylogenetic analysis and sequence alignment

Multiple sequence alignments for the PA biosynthesis genes were generated using ClustalW in DNAMAN, and the phylogenetic tree was constructed with the neighbor-joining algorithm in MEGA (version 10.1) (Tamura et al., 2011). Domains were identified with EXPASY (https://prosite.expasy.org/scanprosite/).

2.3. Chromosomal location, gene structure, and subcellular localization analysis

Each PA synthesis gene was mapped to the tomato genome by identifying their chromosomal positions according to the SGN database (Tomato Genome Consortium, 2012). Accordingly, the cDNA sequences and corresponding genomic DNA sequences of these genes were obtained, and the exons and introns were identified by comparing the genomic DNA and cDNA sequences using the Gene Structure Display server (GSDS; http://gsds.cbi.pku.edu.cn) (Hu et al., 2015).

2.4. Cis-element prediction for PA synthesis gene promoters

The promoter sequence (2 kb upstream of the 5′UTR) of each PA synthesis gene was downloaded from the SGN website and submitted to the PlantCARE database (http://bioinformatics.psb.ugen.be/webtools/plantcare/html/) for cis-element prediction.

2.5. Plant growth conditions, hormone, and stress treatment

Tomato (*S. lycopersicum* L. cv. Micro-Tom) plants were planted in pots with soil (nutrition soil:vermiculite = 2:1) and cultivated in the glasshouse at the College of Horticulture, South China Agriculture University. The growth conditions were as follows: 300 μmol/m^2/s, 12 h/12 h (light/dark) at 25 °C, and a relative humidity of 70%–80%. The roots, stems, leaves, and fruits were harvested from the adult plants. Flower samples were harvested during the flowering period, including two days before anthesis, during the anthesis stage, and 4 days post-anthesis (DPA). Fruits samples included mature green fruit (MG), breaker stage fruit (Br), two days post-breaker stage fruit (Br + 2), and seven days post-breaker stage fruit (Br + 7). Samplings were repeated three times. Each sample was immediately frozen in liquid nitrogen and stored at −80 °C until use.

To investigate the effects of abiotic, hormone, oxidative stress, and PA treatment, tomato seeds were sown on 1/2 MS medium and cultured in the growth chamber for 5 weeks. The seedlings were then treated with 1/2 MS medium containing 100 μM abscisic acid (ABA), 100 μM gibberellic acid (GA3), 100 μM indole acetic acid (IAA), 100 μM 6-benzylaminopurine (6-BA), 100 μM methyl jasmonate (MeJ), 100 μM salicylic acid (SA), 40% ethephon (ETH), 100 mM H_2O_2, 150 μM methylviologen (MV), 300 mM NaCl, 500 μM Put, 500 μM Spd, 500 μM Spm, or 500 μM T-Spm, respectively. Control plants were mock-treated with 1/2 MS medium only. Shoots (including stem and leaf tissue) were collected from both treated and control plants at 0 h, 1 h, 6 h, and 12 h.