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The *Solanum lycopersicum* auxin response factor *SlARF2* participates in regulating lateral root formation and flower organ senescence

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ABSTRACT

ARF2 as apleiotropic developmental regulator has been reported in *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*). The present study showed *SlARF2* transcripts in all tomato plant tissues but with higher accumulation in flowers. During bud–anthesis stages, *SlARF2* transcripts showed a dynamic expression pattern in sepal, stamen, ovary and petal. Hormone treatment analysis suggested that *SlARF2* transcript accumulation was positively regulated by auxin and gibberellic acid, and negatively regulated by ethylene in tomato seedlings. Phenotypes and molecular analyses of *SlARF2*-upregulated transgenic tomato indicated that *SlARF2* regulated tomato lateral root formation and flower organ senescence may be partially mediated by regulating the gene expression of auxin and ethylene response factors. The data enlarges the functional characterization of *SlARF2* in tomato, and broadens our understanding of auxin signaling in regulating plant growth and development.

1. Introduction

Auxin plays a crucial role in almost all aspects of plant growth and development, including embryo patterning, vascular differentiation, flower development, root and stem growth and organ senescence. However, the molecular mechanisms by which auxin regulates fruit growth and development is mostly unknown. Tremendous advances in our understanding of auxin signaling pathways have been made in recent years. Auxin performs its biological function by modulating expression of genes that ultimately regulate particular developmental processes [1–3]. Previous studies have shown that two transcriptional regulator protein families, the auxin response factors (ARFs) and Aux/IAAs, play vital roles in auxin-mediated growth and development by regulating auxin-responsive transcription [4–6].

The molecular mechanisms of auxin metabolism, homeostasis and perception are well documented in *Arabidopsis* and tomato (*Solanum lycopersicum*) [7–9]. Direct binding of auxin to its receptor TIR1 (or its paralogs, the AFB F-box proteins) promotes SCF^{TIR1}–Aux/IAA binding and enhances the ubiquitin-dependent proteolysis of Aux/IAAs in an auxin-dependent manner [10–12]. Degradation of Aux/IAA protein brings about the de-repression of ARFs and subsequent auxin-responsive gene expression [5,6,13–15]. With developments in molecular biology, several components of auxin signaling have been demonstrated to participate in controlling tomato fruit development, including Sl-TIR1 [16], Sl-IAA9 [17], Sl-IAA17 [18],Sl-IAA27 [19], Sl-ARF4 [20] and Sl-ARF7 [21,22].

The involvement of ARF2 in plant developmental processes has been investigated previously. Compared with wild type plants, *arf2* mutants of *Arabidopsis thaliana* displayed pleiotropic developmental phenotypes, including large, dark green rosette leaves, late flowering, thick and long inflorescence, abnormal flower morphology and sterility in early formed flowers, large organ size and delayed senescence and abscission [23–25]. Tomato has long served as a major model for fleshy climacteric fruit development and ripening studies. Two recent studies have showed that *SlARF2* controls in tomato fruit ripening, and over-expressing *ARF2* in tomato resulted in blotchy ripening in which certain fruit regions turn red and possess accelerated ripening and down-regulation of SlARF2 resulted in ripening defects [26,27]. Moreover, it was suggested that in tomato and *Arabidopsis*, ARF2 activity is modulated by Aux/IAA3 and is associated with the auxin–ethylene crosstalk [28,29]. These studies provide a more comprehensive view of ARF2 biological roles.

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Here, *SlARF2* expression was assessed using semi-quantitative PCR and quantitative PCR (Q-PCR). *SlARF2* transcripts existed in all tomato plant tissues but with higher accumulation in flowers. During bud– anthesis stages, *SlARF2* transcripts showed a dynamic expression pattern in sepal, stamen, ovary and petal. Hormone treatment analysis suggested that *SlARF2* transcript accumulation was positively regulated by auxin and gibberellic acid (GA), and negatively regulated by ethylene in tomato seedlings. A *SlARF2* overexpressing Micro-Tom line was obtained by *Agrobacterium tumefaciens* mediated transformation. Phenotypes and molecular analyses of *SlARF2*-upregulated transgenic tomato indicated that *SlARF2* regulated tomato lateral root formation and flower organ senescence may partially mediated by regulating the gene expressions of auxin and ethylene response factors.

2. Materials and methods

2.1. Plant materials and growth conditions

1/2 Murashige and Skoog (1/2 MS) with 0.8% (w/v) agar, pH 5.9 was used for tomato (*Solanum lycopersicum*, cv Micro-Tom) seeds germination and growth as described in Wang et al. (2005). Tomato seedlings grown in light incubator and growth conditions set as follows: 25/20 °C day/night temperature, 14 h day/10 h night cycle, 80% humidity, 250 mol m^{-2} .s^{-1} intense light. The transgenic plants were transferred to soil and grown under standard greenhouse conditions [30]. Different tissues including root, stem, leaf, flower, and fruit were collected from 10-week-old tomato plants. The parts of flower (ovary, stamen, petal and sepal) were acquired from bud (-2 dpa, day post anthesis), anthesis (0 dpa), post-anthesis (4 dpa) and post-anthesis (8 dpa) flowers, respectively.

2.2. Phylogenetic analyses

Phylogenetic analyses were performed with MEGA program (version 3.1) based on amino acid sequences alignment. GenBank accession numbers for the sequences analyzed are as follows: *Arabidopsis thaliana AtARF1* (AEE33612.1), *Arabidopsis thaliana AtARF2* (AED97549.1), *Arabidopsis thaliana AtARF11* (AEC10716.1), *Arabidopsis thaliana AtARF18* (AEE80264.1), *Arabidopsis thaliana AtARF5* (AEE29906.1), *Arabidopsis thaliana AtARF19* (AEE29819.1), *Arabidopsis thaliana AtARF4* (AED97332.1), *Arabidopsis thaliana AtARF9* (AEE84836.1), *Arabidopsis thaliana AtARF6* (AEE31207.1), *Arabidopsis thaliana* AtARF8 (AED94138.1), *Arabidopsis thaliana AtARF10* (AEC08110.1), *Arabidopsis thaliana AtARF16* (AEE85718.1), *Arabidopsis thaliana AtARF17* (AEE36034.1), *Arabidopsis thaliana AtAUX1* (NP_565882.1), *Arabidopsis thaliana AtARF13* (AEE31681.1), *Arabidopsis thaliana AtARF15* (AEE31805.1), *Arabidopsis thaliana AtARF21* (AEE31710.1), *Arabidopsis thaliana AtARF14* (AEE31809.1), *Arabidopsis thaliana AtARF22* (AEE31708.1), *Arabidopsis thaliana AtARF12* (AEE31695.1), *Arabidopsis thaliana AtARF23* (AEE32008.1), *Arabidopsis thaliana AtARF20* (AEE31771.1), *Arabidopsis thaliana AtARF3* (ADB96337.2), *Arabidopsis thaliana AtARF7* (AAD02218.1), *Solanum lycopersicum SlARF19* (NP_001233765.1), *Solanum lycopersicum* SlARF6 (ACU30063.2), *Solanum lycopersicum SlARF4* (NP_001233771.1), *Solanum lycopersicum SlARF19* (NP_001234740.1).

2.3. Ethylene and auxin treatment

The 10-day-old light-grown tomato seedlings germinated and incubated in 1/2 MS buffer as described by Wang et al. [17]. Tomato seedlings were soaked in liquid MS medium with 10 μM NAA for 30 min, 10 μM GA for 2 h and 15 μM Ethephon for 5 h, respectively. The corresponding mock treatment was run concomitantly. Treated seedlings were collected and frozen in liquid nitrogen immediately and stored at −80 °C until RNA isolation. Each treatment was performed in replicate.

2.4. Isolation of the SlARF2 genomic clone

SlARF2 genomic clone was isolated by PCR amplification on genomic DNA template using primers encompassing the coding sequence. DNA sequences was analyzed with BLAST algorithm at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/) [31], and regulatory elements of *SlARF2* promoter were predicted by PlantCARE [32].

2.5. Plant transformation

To generate *35S:SlARF2* transgenic plants, the coding sequence amplified from the tomato cDNA (forward 5′-CGCGGATCCATTAGTGTACGGAAATGGCTGC-3′ and reverse 5′-GGCTCTAGACGT- GATCCTAAGATTCTGCTTG-3′ primers) was inserted into binary vector pCambia-*1301* under the *CaMV 35S* promoter. *Agrobacterium tumefaciens* mediated transformation was used for obtaining transgenic plants according to Wang [17] and Ren [16]. Transformed lines were selected on kanamycin firstly (100 mg L-1) and then screened by both PCR and GUS staining. Homozygous lines from F2 or later generations were used for further analysis.

2.6. Vegetative growth phenotype analyses

The difference of vegetative growth phenotype, such as plant height and lateral root formation, were investigated between wild type and *35S:SlARF2* lines. Eight plants from 2 transgenic lines and wild type were chosen for each line at the 60-day-old tomato, and assessed for plant height (assessed with a ruler). For lateral root formation, six plants at 15-day-old were selected for each tomato line. Given the obvious lateral root formation phenotypes in transgenic lines, the number of lateral root was counted by visual observation. For hormone treatment assay, wild type plant germinated and incubated in MS buffer (10 μM NAA, 10 μM silver nitrate, an inhibitor of the physiological action of ethylene, and 10 μM Ethephon, respctively) for 10 days, six plants were chosen for each treatment group, and counted the number of lateral root.

2.7. Tomato shape phenotyping

For Statistical analyses of the number of pear-shape tomato formation, thirty fruits from two transgenic lines (35S:SlARF2-1, 35S:SlARF2-2) were chosen for each line at 75-day-old immature green fruit stage, and the number of pear-shaped fruit were calculated (assessed by ruler and string). 102-day-old *SlARF2* overexpression lines with conspicuous pear-shaped fruit were selected and photographed.

2.8. Real-time quantitative PCR

Quantitative PCR (Q-PCR) analyses were carried out as described previously [16]. Total RNA was isolated using trizol (Invitrogen, USA) and treated with DNase I (Fermentas, UK), and then purified through Phenol/chloroform. 2 μg of total RNA was used as template to synthesis the first-strand cDNA synthesis by Fermentas Reverse Transcription Kit. According to the protocol as described previously [16,33], Q-PCR was performed to investigate the transcript accumulation of auxin-and ethylene-response factor genes, and the expression of the ACT gene was used as an endogenous control for Q-PCR. All Q-PCR experiments were performed in triplicates