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The Jasmonate ZIM-domain protein gene *SlJAZ2* regulates plant morphology and accelerates flower initiation in *Solanum lycopersicum* plants

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ABSTRACT

JAZ (Jasmonate ZIM-domain) proteins are important repressors in JA signaling pathway. JAZs were proved taking part in various development processes and resistance to biotic and abiotic stresses in *Arabiodopsis*. However, in tomato, the functional study of *JAZs* is rare, especially on plant growth and development. Here, a typical tomato JAZ gene, *SlJAZ2* was isolated. Tomato plants overexpressing *SlJAZ2* exhibited quicker leaf initiation, reduced plant height and internode length, decreasing trichomes, earlier lateral bud emergence and advanced flowering transition. Further experiments showed that the pith cells in transgenic plant stem were much smaller than wild-type and the genes related to cell elongation and gibberellin biosynthesis were down-regulated. Genes mediating trichome formation were also inhibited in plant stem epidermis. In addition, the flower initiation of transgenic plants were earlier and genes controlling flowering time were up-regulated significantly after *SlJAZ2* was overexpressed. Our research demonstrates that *SlJAZ2* accelerates the transition from vegetative growth to reproductive growth.

1. Introduction

In 2000, a novel gene encoding a zinc-finger protein was identified and named as ZIM (Zinc-finger protein expressed in Inflorescence Meristem) in *Arabidopsis* [1]. Subsequently, similar proteins equipped with conserved TIFY (TIF[F/Y]XG) (previously annotated as ZIM domain) amino acid pattern were grouped together in a new plant-specific transcription factor family, TIFY [2]. The TIFY super-family can be classified into four groups: TIFY, PPD (PEAPOD), JAZ (Jasmonate-ZIM) and ZML (ZIM-like) subfamily based on their domain architecture according to the survey on 14 genomes [3]. Among JAZ subfamily, members share two conversed motifs, ZIM and Jas (also known as CCT_2). TIFY domain mediates homo- and heteromeric dimerization among JAZs [4,5] and the interaction of JAZs with Groucho/Tup1-type co-repressor NINJA (Novel Interactor of JAZ) to recruit TPL (TOPLESS) and TPRs (TPL-related proteins), proteins that function as negative regulators of jasmonate responses [6]. Besides, few JAZs directly recruit TPL through an conversed EAR motif present in the N terminal [7]. The C terminal Jas motif is specific of the JAZ family and induces the interaction of JAZs with JA (Jasmonic acid) receptor, the F-box COI1 protein and most TFs it represses [8].

Recently JAZ subfamily members in many species were identified and isolated. In *Arabidopsis*, 12 canonical proteins are included in JAZ subfamily [3]. In other species, JAZ proteins were also searched and investigated in the whole genome [9–15]. Certain JAZs serve as the repressors of JA signaling pathway and have been proved to be the targets of Skp/Cullin/F-box (SCF^{COI1}) complex and degraded through the 26 S proteasome pathway [16]. Previous studies revealed that JA is an important plant hormone participating in plant defense. Therefore, the extensive researches on *JAZ* genes mainly impact plant defense to abiotic and biotic stresses via JA pathway. For example, overexpression of *Arabidopsis JAZ1* or *JAZ4* attenuating freezing stress responses in plants by repressing the ICE-CBF/DREB1 (INDUCER of CBF EXPRESSION-DRE-C-REPEAT-BINDING FACTOR BINDING FACTOR1) pathway [17], while *JAZ7* is induced by *Fusarium oxysporum* and ectopic *JAZ7* expression in *Arabidopsis* increases susceptibility to *F. oxysporum* due to

Abbreviations: ZIM, zinc-finger protein expressed in inflorescence meristem; TIFY, TIF[F/Y]XG; JAZ, jasmonate-ZIM; ZML, ZIM-like; NINJA, novel interactor of JAZ; TPL, topless; TPRs, TPL-related proteins; ICE-CBF/DREB, inducer of CBF expression-DRE-c-repeat-binding factor binding factor1; TOE, target of eat; FT, flowering locus T; WT, wild-type; IMG, immature green at 20 DPA; DPA, day post anathsis; MG, mature green at 35 DPA; B, breaker with the color change from green to yellow; B4, 4 days after breaker; B7, 7 days after breaker; PRE, paclobutrazole resistance; HLH, helix-loop-helix; CPS, copalyl diphosphate synthase; Wo, woolly; BL, blind; FA, falsiflora; SFT, single flower truss; UF, uniflora

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the disturbed finely-tuned COI1-JAZ-TPL-TF complexes in JA signaling pathway [18]. Moreover, JAZs work on plant growth and development. *JAZ7* is induced by darkness and loss-function-of *JAZ7* accelerates leaf senescence in darkness [19]. Several JAZs interact with WD-repeat/bHLH/MYB complexes and subsequently activate downstream signal cascades to modulate anthocyanin accumulation and trichome initiation [20].

Sufficient studies on *Arabidopsis* JA-biosynthesis and JA-signaling mutants have established that JA also performs a critical role in plant male fertility [21–24]. As vital components of JA signaling pathway, JAZs are confirmed involved in plant reproductive development. Elevated *JAZ7* transcription leads to an early flowering phenotype in *Arabidopsis* [18]. The similar phenotype is also observed in *AtJAZ1ΔJas* (Jas-domain deletion) overexpression plants. AtJAZ1 interacts with two AP2 transcription factors TOE1 (TARGET OF EAT1) and TOE2 which repress the transcription of *FT* (*FLOWERING LOCUS T*), a vital gene taking part in the floral determination, so that the flowering time is regulated by AtJAZ1 indirectly [25]. In addition, Arabidopsis JAZ1, JAZ8 and JAZ11 target MYB21 and MYB24, two R2R3-MYB transcription factors which are required for stamen development to modulate male fertility specifically [26]. Under stress condition, *PpJAZ1* mediates the transition of peach flowers from out crossing to self-pollination [27]. In *Nicotiana attenuata* plants, silencing JAZ subfamily protein gene *NaJAZd* produced fewer capsules due to increased flower abscission in later stages of flower development indicating that *NaJAZd* is required to counteract flower abscission [28]. OsJAZ1 interacts with OsMYC2 to repress its role in activating *OsMADS1*, an E-class gene and finally regulates the spikelet development [29].

So far, 12 JAZs have been identified in tomato [15]. Previous studies showed that most *SlJAZs* are JA and JA-isoleucine mimic induced, and responsive to one or more abiotic stresses [15,30]. In addition, *SlJAZ2*-, *SlJAZ6*- and *SlJAZ7*-silenced tomato plants showed enhanced disease-associated cell death to *Pst* DC3000 (*P. syringae* pv. *tomato* DC3000) [30]. However, the experimental evidence of *SlJAZs* implicated in tomato plant development has been barely provided. Here we report the characterization of *SlJAZ2* (accession number: Solyc12g009220), a typical JAZ subfamily protein gene homologous to *AtJAZ1* (accession number: At1g19180) which was reported to participate in the regulation of flowering time. To further explore the biotic function of *SlJAZ2* in the growth and reproductive development of tomato plants, we obtained the transgenic tomato plants with *SlJAZ2* overexpressed. And we found that overexpression of *SlJAZ2* alters tomato plant architecture at early stage and accelerates flower initiation. Theses phenotypes were further verified by morphological, statistic and molecular analysis. Our research extends the knowledge of *JAZ* family genes modulating the processes of plant early vegetative growth and transition to reproductive stage.

2. Materials and methods

2.1. Plant materials and growth condition

The tomato AC^{++} (*Solanum lycopersicum* Mill. cv. Ailsa Craig) was used as WT (wild-type) in our study. The transgenic and wild-type tomato plants were planted under standard greenhouse conditions (16-h-day/8-h-night cycle, 25 °C/18 °C day/night temperature, 80% humidity, and 250 μmol $m^{-2} s^{-1}$ light intensity). The transgenic tomato plants of the first generation (T0) came from tissue culture. The tomato plants used in this study were the second generation (T1) which came from seeds. For expression pattern analysis, flowers were tagged at anathsis. The stages of tomato fruits were divided into IMG (immature green at 20 DPA (day post anathsis)), MG (mature green at 35 DPA), B (breaker with the color change from green to yellow), B4 (4 days after breaker) and B7 (7 days after breaker). The Supplementary Fig. S1 showed different stages of tomato fruits. All plant samples were immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. Total RNA extraction and quantitative real-time PCR analysis

Total RNA of different tissues were extracted using RNAiso Plus (Takara) according to the manufacturer's instructions. Total RNA (1 μg) was used for the following experiment of first strand cDNA synthesis catalyzed by M-MLV reverse transcriptase (Promega) with tailed Oligo $d(T)_{18}$ primer (Supplementary Table S1). cDNA was conserved at −20 °C.

Quantitative real-time PCR was performed on the CFX96™ Real-Time System (Bio-Rad) using the GoTaq qPCR Master Mix (Promega). *SlCAC* and *SlActin-51* were used as internal reference gene for qPCR studies during tomato development process [31]. For each gene analysis, NRT (no reverse transcription control) and NTC (no template control) were performed. Relative quantification of gene transcription level was analyzed using $2_T^{-\Delta\Delta C}$ method [32]. The gene-specific primers for qPCR analysis are listed in Supplementary Table S1. Analyses of gene expression were performed in three biologic repeats.

2.3. Construction of SlJAZ2 over-expression vector and plant transformation

We obtained the cDNA sequence of *SlJAZ2* (Solyc12g009220) in Sol Genomics Network (https://solgenomics.net/). SlJAZ2-F and SlJAZ2-R (Supplementary Table S1) primers tailed with *Bam*H I and *Sac* I restriction sites at the 5′ end respectively were designed to amplify the ORF of *SlJAZ2*. The cDNA of wild-type tomato was used as template for *SlJAZ2* gene amplification. The amplified products were digested and linked into the plant binary vector pBI121. The vector carried CaMV 35S promoter-*SlJAZ2* expression unit was transferred into *Agrobacterium* LBA4404 strain. Subsequently, positive LBA4404 strain was transferred into WT tomato cotyledon explants through *Agrobacterium*-mediated transformation method [33]. The transgenic tomato plants were selected on kanamycin medium and detected by PCR with primers NPT II-F and NPT II-R (Supplementary Table S1).

2.4. Measurement of plant architecture parameters and flowering time

To differentiate between WT and *SlJAZ2*-OE (over-expression) transgenic tomato plants, we measured the height, leaf number and internode length respectively every week from 21 days after germination. In order to understand the difference of transgenic lines in the transition process from vegetative growth to reproductive growth, the days to visible flower and leaf number under the first reproductive structure were measured. For each data of WT and transgenic lines, at least ten plants were measured.

2.5. Anatomical and cytological analyses of the stems

The stems from cotyledon to the first true leaf were fixed in FAA (70% ethanol/acetic acid/formaldehyde (18:1:1)). Then the dehydration, fixation, sectioning, dewaxing and staining by safranin and fast green were performed to prepare the longitudinal sections of WT and transgenic plant stems. The pith cells were visualized under a microscope (OLYMPUS IX71) and photographed.

2.6. Statistical analysis

Data were presented as mean ± standard deviation. Significant difference between transgenic lines and WT was analyzed using Student's *t*-test ($P < 0.05$). The measurement values showed came from the means of three biological replicates.