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The MADS-box gene *SlMBP11* regulates plant architecture and affects reproductive development in tomato plants

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ABSTRACT

MADS-domain proteins are important transcription factors that are involved in many biological processes of plants. In the present study, *SlMBP11*, a member of the AGL15 subfamily, was cloned in tomato plants (*Solanum lycopersicon* M.). *SlMBP11* is ubiquitously expressed in all of the tissues we examined, whereas the *SlMBP11* transcription levels were significantly higher in reproductive tissues than in vegetative tissues. Plants exhibiting increased *SlMBP11* levels displayed reduced plant height, leaf size, and internode length as well as a loss of dominance in young seedlings, highly branched growth from each leaf axil, and increased number of nodes and leaves. Moreover, overexpression lines also exhibited reproductive phenotypes, such as those having a shorter style and split ovary, leading to polycarpous fruits, while the wild type showed normal floral organization. In addition, delayed perianth senescence was observed in transgenic tomatoes. These phenotypes were further confirmed by analyzing the morphological, anatomical and molecular features of lines exhibiting overexpression. These results suggest that *SlMBP11* plays an important role in regulating plant architecture and reproductive development in tomato plants. These findings add a new class of transcription factors to the group of genes controlling axillary bud growth and illuminate a previously uncharacterized function of MADS-box genes in tomato plants.

1. Introduction

MADS-domain proteins are important transcription factors participating in a variety of plant developmental processes, such as in *Arabidopsis*, including the regulation of floral organ identity [1–5], inflorescence meristem identity [6–9], flowering time control [10–16], formation of the dehiscence zone [17], fruit ripening [17,18], seed pigmentation, embryo development [19], and development of vegetative organs, i.e., roots and leaves [20–23]. In tomato plants, the *MACROCALYX* (*MC*) gene is involved in the development of sepals in the first whorl and in inflorescence determinacy [24]. *TPI*- and *SlGLO1*-silenced plants exhibit aberrant carpelloid stamens [25,26]. A mutation in *TAP3* and the silencing of *TM6* both result in the conversion of stamens into carpels and a more or less severe conversion of petals into sepals [27]. The MADS domain protein JOINTLESS is necessary to specify pedicel abscission zones in tomatoes. Abscission zones are absent in the *jointless* mutant [28]. In addition, *MADS-RIN* and *TOMATO AGAMOUS-LIKE 1* (*TAGL1*) control fruit ripening [24,29]. Recently, we found that the tomato MADS-box transcription factor SlMADS1 acts as a negative regulator of fruit ripening and interacts with SlMADS-RIN [30]. It was also found that the overexpression of the MADS-box gene *SlFYFL* delays senescence, fruit ripening and abscission in tomatoes [31].

Members of the MADS-box gene family are found throughout higher eukaryotes and are divided into two lineages, Type I and Type II, which arise from single gene duplication before the divergence of plants and animals [21]. Genes from the type I lineage can be further classified into three subclasses: Mα, Mβ and Mγ [32]. Type II genes in plants are termed MIKC (MADS/intervening/keratin-like/C-terminal) genes in reference to the four recognized domains of their protein products; MIKC-type genes have been further classified into MIKC^{C} and MIKC* types based on differences in their domain structure [33]. MIKC^{C} genes are the best-studied plant MADS-box family and are divided into at least 13 subfamilies based on their phylogeny, such as the FLOWERING LOCUS C (FLC), SQUAMOSA (SQUA), AGL6, SEPALLATA (SEP)/AGAMOUS-LIKE 2 (AGL2), TM8, TOMATO MADSBOX 3 (TM3)/SOC1, AGL17, AGL15, SOLANUM TUBEROSUM MADS-BOX 11 (STMADS11), AGL12, AGAMOUS (AG), Bsister (GGM13), and

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DEFICIENS (DEF)/GLOBOSA (GLO) subfamilies [34]. All members of this family contain a highly conserved motif of 55–60 amino acids, known as the MADS domain, which is essential for the DNA-binding activity of these factors [35]. Many of the family members identified in plants also contain a less-conserved domain called the K domain, which may facilitate protein-protein interactions. The C domain, which constitutes approximately the last one-third of each protein, has a more variable sequence and is likely to contribute to the differences in function. Among them, the AGL15 (AGAMOUS-like 15) subfamily is a member of the MIKC^{C}-type MADS-box genes. In *Arabidopsis*, overexpression of the *AGL15* gene delays flowering, fruit maturation, floral organs senescence and abscission, suggesting that this MADS-box transcription factor is a negative regulator of these processes [36,37]. The MADS-domain transcriptional regulator AGAMOUS-LIKE15 promotes somatic embryo development in *Arabidopsis* and soybeans [38]. Analysis of transgenic *Arabidopsis* plants showed that the overexpression of *AGL18*, a member of the AGL15-like clade of MADS domain regulatory factors, produces the same phenotypic changes, such as overexpression of *AGL15*, and the two genes have partially overlapping expression patterns. Functional redundancy was confirmed through the analysis of loss-of-function mutants. The *agl15 agl18* double mutants flower early under non-inductive conditions, indicating that *AGL15* and *AGL18* act in a redundant fashion as repressors of the floral transition [39].

In a previous study, we reported the important role of *SlMBP11* as a stress-responsive transcription factor in the positive modulation of salt stress tolerance in tomato plants [40]. However, studies on the development of related functions of the MADS-box transcription factor SlMBP11 in tomato plants have not been reported. To comprehensively examine the diversified functions of MADS-box genes in tomato plants, we have isolated the *SlMBP11* gene from wild-type tomato (*Solanum lycopersicon Mill. cv. Ailsa Craig*) flowers and carried out a detailed expression analysis. We succeeded in generating visible *SlMBP11* overexpression lines. Overexpression of *SlMBP11* in tomato plants not only altered plant architecture but affected the reproductive structure. Moreover, our results also revealed that overexpression of *SlMBP11* in tomato plants delays perianth organ senescence. These phenotypes were further confirmed by analyzing morphological, anatomical and molecular features of plants exhibiting overexpression. This article enhanced our knowledge about the roles of *SlMBP11* in diverse developmental processes.

2. Material and methods

2.1. Plant materials and growth conditions

In our experiments, we used tomato plants (*Solanum lycopersicum* 'Ailsa Craig' AC^{++}), a near-isogenic tomato line, as the wild type. The plants were planted in a greenhouse and watered daily. Transgenic cultures grown 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). Plants of the first generation (T0) came from tissue culture, and plants of the second generation (T1) were from seedlings. The third generation (T2) tomato plants were used in the experiments. Flowers were tagged at anthesis. The ripening stages of tomato fruits were divided according to the days post anthesis (DPA) and fruit color. In the wild type, immature green fruits were defined as 20 DPA. Mature green fruits were defined as 35 DPA and characterized as being green and shiny with no obvious color change. Breaker fruits were defined as fruits of 38 DPA with the color change from green to yellow. Other fruits of 4 d after breaker and 7 d after breaker were also used. All plant samples were immediately frozen with liquid nitrogen, mixed, and stored at −80 °C until further use.

2.2. SlMBP11 isolation

The total RNA of the tomato was extracted using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. Then, 1 μg of total RNA was used to synthesize first strand cDNA through reverse transcription polymerase chain reaction (M-MLV reverse transcriptase, Takara) with tailed Oligo d(T)$_{18}$ primer (Supplementary Table S1). cDNA (1–2 μl) was used to clone the full length of the *SlMBP11* gene with SlMBP11-F and SlMBP11-R primers (Supplementary Table S1) through high-fidelity PCR (Prime STARTM HS DNA polymerase, Takara). The amplified products were tailed by using a DNA A-Tailing kit (Takara) and linked with pMD18-T vector (Takara). Positive clones were picked out via Escherichia coli JM109 transformation and confirmed by sequencing (BGI, China).

2.3. Construction of the SlMBP11 overexpression vector and plant transformation

The above-mentioned MBP11-pMD18-T vector was used as the template and amplified with SlMBP11-F and SlMBP11-R primers tailed with *Bam*H I and *Sac* I restriction sites at the 5′ end, respectively. The amplified products were digested with *Bam*H I and *Sac* I, respectively, and linked into the plant binary vector pBI121 with *Sac* I and *Bam*H I restriction sites. The transgene was under the control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter. The generated binary vectors were transferred into *Agrobacterium* LBA4404, and *Agrobacterium*-mediated transformation was performed following the protocols described by Chen et al. [41]. The transgenic plants were detected with NPTII-F (5′-GACAATCGGCTGCTCTGA-3′) and NPTII-R (5′-AACTCCAGCATGAGATCC-3′) primers. The positive transgenic plants were selected and used for subsequent experiments.

2.4. Quantitative real-time PCR analysis

Total RNAs extraction and cDNA synthesis of wild type and transgenic lines were performed as described above. Quantitative RT-PCR was performed using the CFX96^{TM} Real-Time System (C1000^{TM} Thermal Cycler, Bio-Rad, USA). All reactions were carried out using the SYBR Premix Ex Taq II kit (Takara) in a 10 μl total sample volume (5.0 μl of 2×SYBR Premix Ex Taq, 1.0 μl of primers, 1.0 μl of cDNA, and 3 μl of distilled deionized water). To remove the effect of genomic DNA and the template from the environment, no-template control and no-reverse transcription control experiments were performed. Additionally, three replications for each sample were used, and standard curves were run simultaneously. Tomato *SlCAC* [42] was used as an internal standard. The primers SlMBP11-Q-F and SlMBP11-Q-R were used to determine the expression levels of *SlMBP11* in the wild type and transgenic lines. All primers used for quantitative RT-PCR are shown in Supplementary Table S1.

2.5. Expression analysis of SlMBP11 by gene microarray

Microarray expression data were obtained using Genevestigator (https://www.genevestigator.com/gv/) with the tomato Gene Chip platform. The nucleotide sequence of *SlMBP11* was used as the query sequence to blast against all of the gene probe sequences from the Affymetrix Gene Chip (http://www.affymetrix.com/), and the best homologous probe (Les.2212.1.A1_at) was selected and used to perform search in the Affymetrix Tomato Genome Array platform of Genevestigator.

2.6. Measurement of plant architecture parameters

To understand the differences between wild type and transgenic lines, we measured the height, node number, internode length, and