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Alteration of *SlYABBY2b* gene expression impairs tomato ovary locule number and endogenous gibberellin content*#

Hui LI§†, Mei-hua SUN§, Ming-fang QI, Jiao XING, Tao XU, Han-ting LIU, Tian-lai LI†‡

Key Laboratory of Protected Horticulture of Liaoning Province / Collaborative Innovation Center of Protected Vegetable Surround Bohai Gulf Region / Horticulture Department, College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China

†E-mail: 2671329915@qq.com; tianlaili@126.com

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Abstract: Tomato is an ideal model species for fleshy fruit development research. *SlYABBY2b* regulates the ovary locule number, which is increased by gibberellins, in tomato. However, the relationship between *SlYABBY2b* and endogenous gibberellin is poorly understood. In this study, *SlYABBY2b*-overexpressing and RNA interference (RNAi) transgenic tomato plants were used to elucidate the mechanism by which *SlYABBY2b* regulates the ovary locule number and endogenous gibberellin content in tomato. *SlYABBY2b*-overexpressing plants showed fewer locules and lower gibberellin content than the control plants. Contrasting results were found in the RNAi lines. Therefore, the *SlYABBY2b* gene negatively regulates tomato ovary locule number and endogenous gibberellin content. Furthermore, the expression of *SlYABBY2b* gene was remarkably higher than that of the wild type in the apical shoots of gibberellin-deficient mutants. This showed that the gibberellins can inhibit the expression of *SlYABBY2b* gene negative regulation. Further study revealed that *SlYABBY2b* suppressed the expression of *SlGA20ox1* and *SlGA3ox2*, but increased that of *SlGA2ox1* and *SlGA2ox5* in the apical shoots of *SlYABBY2b*-overexpressing plants, thereby reducing gibberellin content. Contrasting results were found in the RNAi lines. Our results showed that the *SlYABBY2b* gene was located on gibberellin signal transduction pathways, fed back regulation of the synthesis of gibberellin, and felt exogenous gibberellin signal to further regulate the formation of tomato locule.

Key words: *SlYABBY2b*; Gibberellin; Gibberellin-deficient mutants; Ovary locule number; Transgenic plant; Gene expression; Tomato

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1 Introduction

Tomato is an ideal model species for fleshy fruit development research. Domesticated tomato fruit is enlarged 1000 times compared to their wild progenitors, which is an extreme case. Fruit size and weight are the primary characteristics of commercial tomato varieties. Increases in fruit weight and fruit size are controlled by multiple quantitative trait loci (QTL), and some of them have been cloned or identified, namely, six fruit weight QTLs and three locule number QTLs (*fw2.2*, *fw3.2*, *fw1.1*, *fw3.3*, *fw6.1*, *fw11.2*, *lcn2.1*, *lcn2.4*, *lcn5.1*) (Fukazawa et al., 2000; Foolad, 2007; Chakrabarti et al., 2013; van der Knaap et al., 2014; Fernández-Lozano et al., 2015; Hernández-Bautista et al., 2015; Illa-Berenguer et al., 2015). Locule number is associated with fruit size and weight, and almost all wild species and several

‡ Corresponding author
§ The two authors contributed equally to this work
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ORCID: Hui LI, https://orcid.org/0000-0002-7151-5912

small-fruited varieties produce fruits with only 2–4 locules, whereas most cultivar varieties consumed today can develop fruits with up to 10 locules. Increased locule number contributes as much as 50% variance to fruit enlargement and is believed to represent the second major step in the tremendous increase in tomato fruit size in evolution (Lippman and Tanksley, 2001; Tanksley, 2004). Molecular genetic studies have so far in tomato identified two traits that govern fruit locule number, QTL *fasciated* and QTL *lc*, which control floral meristem size and ultimately the development of supernumerary locules (Lippman and Tanksley, 2001; Barrero and Tanksley, 2004; Barrero et al., 2006; Muños et al., 2011; van der Knaap et al., 2014; Xu et al., 2015). Both of these traits have an effect on the shape and fruit size in the tomato (Muños et al., 2011; Rodríguez et al., 2011). Regarding the *lc* locus, which has a weaker effect, located in a 1608-bp non-coding region, two single-nucleotide polymorphisms (SNPs) are responsible for the extreme high-locule-number phenotype (Muños et al., 2011). Two SNPs were proposed to disrupt the repression of *SlWUS* by *TOMATO AGAMOUS-LIKE1* (*TAG1*), which is the homolog of the MADS-box gene of *Arabidopsis* flower development *AGAMOUS* (van der Knaap et al., 2014). *SlWUS* and peptide *SlCLAVATA3* (*SlCLV3*) interact in a negative feedback loop (Schoof et al., 2000). Recent studies have shown that down-regulation of *SlWUS* affected tomato flower and locule development (Li et al., 2017). The *fasciated* mutation contains a 294-kb inversion with two breakpoints, named the first intron 1 of *SlYABBY2b* and upstream of the tomato *SlCLV3* start codon (Huang and van der Knaap, 2011), resulting in reduced messenger RNA (mRNA) accumulation of the *SlCLV3* gene (Xu et al., 2015). *SlYABBY2b*, one of the 9 *Solanum lycopersicum SlYABBY* genes (Huang et al., 2013), is the first found gene encoding a *YABBY*-like transcription factor that controls fruit locule number development in tomato (Cong et al., 2008).

Increases in fruit weight and fruit size are controlled by tomato fruit locule number. However, the tomato fruit locule number is strongly linked to malformation. Fruits with several ovary locules and large sizes are prone to malformation. Fruit malformation is affected by the environment and hormones (Asahira et al., 1982; Tomer et al., 1998; Li et al., 2008), and gibberellin (GA) content has also been associated with fruit malformation (Sawhney and Greyson, 1971; Sawhney and Dabbs, 1978; Liu and Li, 2012).

Fruit development is a complex and precise genetically regulated process (Seymour et al., 2013). Plant hormones play a key role in controlling fruit growth and development in the tomato. GAs are growth factors that participate in the process of plant growth and development (Olszewski et al., 2002; Kumar et al., 2014; Pesaresi et al., 2014), and the metabolic pathway of the plant is well understood. GA levels are influenced by GA biosynthesis and inactivation (Hedden and Phillips, 2000). These enzymes, named *GA20ox*, *GA3ox*, and *GA2ox*, involved in the GA metabolism pathway, are encoded by small gene families and regulate bioactive GA levels (Hedden and Phillips, 2000; Sakamoto et al., 2004; Yamaguchi, 2008). Many transcription factors, including those from the YABBY family, are also involved in regulating GA biosynthesis in plants (Fukazawa et al., 2000; Rosin et al., 2003; Gazzarrini et al., 2004; Ishida et al., 2004; Magome et al., 2004; Wang et al., 2004; Dai et al., 2007), and thus in controlling the growth and development of plants. YABBY transcription factors are closely linked to GA synthase genes in *Arabidopsis* and rice (Hay et al., 2002; Kumaran et al., 2002; Dai et al., 2007).

SlYABBY2b, which was the first YABBY transcription factor identified in tomato, regulates fruit development by increasing locule number. GAs also play a similar role in fruit development. However, the relationship between *SlYABBY2b* and endogenous GA is poorly understood. In the present study, we analyzed the regulatory effects of *SlYABBY2b* on tomato ovary locule number and endogenous GA content through transgenic approaches.

2 Materials and methods

2.1 Plant materials

Tomato (*Solanum lycopersicum* L.) lines MLK1 and FL1 were used in the research. The “MLK1” line, which has multiple locules, and the “FL1” line, which has few locules, were obtained from Shenyang Agricultural University, Shenyang, China. The other agronomic characteristics are similar, except for the differences in locule number. The locule numbers of

MLK1 and FL1 are about 14 and 2, respectively. All *gib* mutants and wild types were obtained from the University of California, Davis, USA. Plants were grown in soil under greenhouse growing conditions (25 °C day, 15 °C night) in September 2015 at Shenyang Agriculture University. *gib* mutants and wild types are listed in Table S1.

2.2 Construction of *SlYABBY2b* RNAi and overexpressing vectors, and tomato transformation

A 551-bp fragment of *SlYABBY2b* was amplified from "MLK1" by real-time polymerase chain reaction (RT-PCR). Specific primers were designed using the sequences of the *SlYABBY2b* gene from tomato (Gene Bank accession number EU557674) (Cong et al., 2008). The overexpression- and RNAi-specific primers used for the *SlYABBY2b* gene are as follows: *SlYABBY2b* 1: CACCTCCCCTTTGATCCATGTTCT (forward) and CGCTATTTGTTGCCCTCC (reverse); *SlYABBY2b* 2: TCCCCTTTGATCCATGTTCT (forward) and CGCTATTTGTTGCCCTCC (reverse). The sequence-confirmed amplified fragments were cloned into the Gateway-compatible vector pENTR/D-TOPO and pCR8/GW/TOPO entry vectors via a TOPO cloning reaction (Invitrogen), respectively. The nucleotide sequence was verified, and the fragments were then transferred to the binary vectors pB7WG2D and pB7GWIWG2 via LR recombinant reaction, respectively. Through sequence analysis and restriction enzyme digestion, the constructs containing the expected insert were introduced into *Agrobacterium tumefaciens* LBA4404 cells by electroporation.

Seeds of "MLK1" and "FL1" were surface-sterilized with 70% (v/v) alcohol for about 30 s, sown on 1/2 Murashige and Skoog (MS) medium, and cultured in a 16-h day/8-h night regime at 25 °C for 6–8 d. The explants from tomato seedling cotyledons were cut and pre-cultured on a pre-medium in darkness at 25 °C for 48 h. After pre-culturing, *A. tumefaciens* LBA4404 cells were used to infect the explants for 4 min with slow shaking. After infection, the explants were cultured on a cocultivation medium for 2 d in darkness at 25 °C. Then, the explants were cultured on selective medium, which contained MS salts, 3% (0.03 g/ml) sucrose, 7 g/L agar, 2 mg/L 6-benzylaminopurine (6-BA), 0.2 mg/L indole-3-acetic acid (IAA), 400 mg/L cephalosporin, and 0.5 mg/L glufosinate-ammonium to induce regeneration. Explants that regenerated plantlets were transplanted on a rooting medium including MS salt, 3% (0.03 g/ml) sucrose, 5 g/L agar, 400 mg/L cephalosporin, and 0.05 mg/L 1-naphthlcetic acid (NAA). The plants were grown in soil under greenhouse growing conditions.

2.3 Total RNA extraction and RT-PCR analysis

For quantitative real-time PCR (qRT-PCR), total RNA was extracted using TRIzol® reagent followed by the DNA-free™ kit (Ambion) in accordance with the instructions. Complementary DNA (cDNA) samples were synthesized from 1 μg of RNA using cDNA Archive kit (Life Technologies, USA). qRT-PCR was performed following the method described by Jain et al. (2006). RT-PCR amplification was conducted on an Applied Biosystems 7500 real-time system using SYBR Green PCR Master Mix (Life Technologies, USA) as described in accordance with the instructions. Three independent qRT-PCR analyses were carried out for each cDNA preparation. Data were normalized to actin and the specificity of the reactions was verified by the $\Delta\Delta C_T$ calculation method. The sequence of specific primers used for RT-PCR is listed in Table S2. Significant difference analysis was tested using Duncan's multiple range test, with $P<0.05$ being considered statistically significant. The data were analyzed using Origin 8.0. Analysis of variance (ANOVA) was performed using SPSS 13.0 software.

2.4 Seedling sampling

Stem apices of seedlings were selected at the same stage. The stem apices of the transgenic and wild-type plants were observed under a microscope during the flower bud differentiation phase. The stem apices of the transgenic and control plants were dissected (30 per generation, 0.3 cm long) in triplicate. The freshly collected samples were immediately frozen in liquid nitrogen and then stored at −80 °C for RT-PCR analysis.

2.5 ELISA analysis of gibberellins

Extraction, purification, and measurement of GAs were performed using an enzyme-linked immunosorbent assay (ELISA) kit as described previously (Zheng and Zhou, 1995; Chen et al., 1998). The apical shoot

samples at different developmental stages were collected in liquid nitrogen and extracted in cold 80% (v/v) ethanol solution. The samples were ground in ethanol solution with 80% (v/v) cold methanol three times in a cold mortar and moved to a suitable container in dark conditions at 4 °C overnight. Then, the supernatant extract was filtered through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA). The extracts (ethyl acetate phases) were collected and dried under nitrogen, and the residue was re-dissolved in phosphate buffer saline (PBS; 0.01 mol/L, pH 7.4). Antibodies against GA were purchased from the Abmart Company (China). A solution of antibodies was coated on microtitration plates and incubated at 37 °C for 90 min. The wells were washed five times with PBS. Each well was measured for absorbency at 490 nm to determine the content of GAs.

2.6 Phenotypic analysis

The stem height and internode length of 12-week-old tomato plants grown in greenhouse conditions were measured. Stem height was measured from the bottom to the top of the tomato plant. Internode length was calculated by dividing the stem height by the leaf number. The numbers of total flowers, sepals, and petals in the first, second, and third inflorescences were counted, and the fruits were weighed. The first, second, and third clusters of three fruits from the transgenic and non-transgenic plants were harvested, and their locule numbers were determined. Significant difference analysis was tested using Duncan's multiple range test, with $P<0.05$ being considered statistically significant. ANOVA was performed using SPSS 13.0 software.

3 Results

3.1 Phenotypic characterization of transgenic plants

Gene-specific PCR analysis revealed that six independent *SlYABBY2b*-overexpressing transgenic plants contained the desired insert (data not shown). All *SlYABBY2b*-overexpressing plants were morphologically distinguishable from the non-transgenic control plants. They exhibited dwarf phenotypes due to the decreased internode lengths (Fig. 1g; Table 1). Both shoot and internode lengths in all transgenic lines were significantly shorter than those of the control (Fig. 1h; Table 1). However, the stem diameter was markedly greater in the *SlYABBY2b*-overexpressing lines than in the control, except at the four-leaf stage (Table 1). The number of flowers in the first and second inflorescences was significantly higher in the *SlYABBY2b*-overexpressing lines than in the control, whereas the number of sepals was significantly lower in the *SlYABBY2b*-overexpressing lines than in the control (Figs. 1a and 1b; Table 1). The number of petals decreased in the transgenic lines (Table 1). The locule number significantly decreased in the overexpressing transgenic plants, especially in the fruits of the first inflorescence (Figs. 1c and 1d; Table 1). Fruit weight was also reduced with decreasing locule number (Figs. 1e and 1f). Five *SlYABBY2b* RNAi transgenic lines were obtained and identified by PCR analysis (data not shown). For further investigation, we selected one line because they have similar phenotypic features within the group. The *SlYABBY2b* RNAi plants had significantly greater plant heights and internode lengths but smaller stem diameters than the control plants (Fig. 2e; Table 2). The number of flowers in every inflorescence was significantly higher than that in the control. The numbers of sepals and petals increased (Table 2). In addition, the locule number and fruit weight of the transgenic plants significantly increased (Figs. 2a–2d; Table 2).

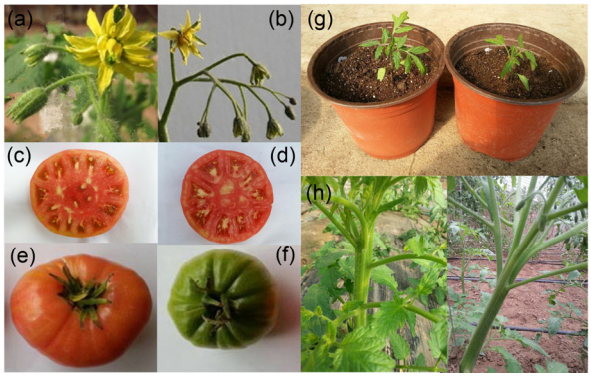

Fig. 1 Phenotypic characteristics of representative wild-type and *SlYABBY2b*-overexpressing (OE) transgenic tomato plants

(a) Flower from MLK1 plants. (b) Flower from *SlYABBY2b*-OE lines. (c) MLK1 locules. (d) *SlYABBY2b*-OE locules. (e) MLK1 fruit size. (f) *SlYABBY2b*-OE fruit size. (g) MLK1 (left) and *SlYABBY2b*-OE (right) plants at same leaf stage. (h) MLK1 (left) and *SlYABBY2b*-OE (left) stems

Table 1 Phenotype of the wild-type and *SlYABBY2b*-overexpressing transgenic plants

Type	Plant height (cm)			Stem diameter (cm)		
	4-leaf stage	8-leaf stage	12-leaf stage	4-leaf stage	8-leaf stage	12-leaf stage
MLK1	5.1±0.2^{bB}	9.6±0.6^{bB}	43.4±3.3^{bB}	0.224±0.017^{aA}	0.395±0.040^{aA}	0.812±0.065^{aA}
SlYABBY2b-OE	4.6±0.4^{aA}	8.3±0.5^{aA}	34.4±2.2^{aA}	0.264±0.038^{aA}	0.474±0.041^{bB}	0.901±0.129^{bB}
Type	The first internodal length (cm)			The second internodal length (cm)		
	4-leaf stage	8-leaf stage	12-leaf stage	4-leaf stage	8-leaf stage	12-leaf stage
MLK1	0.6±0.1^{aA}	1.8±0.4^{bB}	2.9±0.3^{bB}	0.6±0.2^{aA}	1.9±0.3^{bB}	2.8±0.3^{bB}
SlYABBY2b-OE	0.6±0.2^{aA}	1.4±0.3^{aA}	2.1±0.2^{aA}	0.6±0.1^{aA}	1.5±0.2^{aA}	2.0±0.3^{aA}
Type	The first flower of the first truss			The second flower of the first truss		
	First fruit	Second fruit	Third fruit	First fruit	Second fruit	Third fruit
MLK1	16.8±1.5^{bB}	13.2±1.6^{bB}	12.9±1.7^{bB}	14.2±2.5^{bB}	12.6±2.8^{bB}	14.3±1.9^{bB}
SlYABBY2b-OE	10.9±1.7^{aA}	9.9±1.5^{aA}	10.4±1.4^{aA}	11.4±2.9^{aA}	10.6±1.4^{aA}	12.3±1.9^{aA}
Type	The third flower of the first truss			The first inflorescence		
	First fruit	Second fruit	Third fruit	Total flowers	Sepals	Petals
MLK1	14.5±1.7^{bB}	12.1±1.6^{bB}	11.4±1.7^{aA}	5.3±1.5^{aA}	8.6±0.7^{bB}	8.9±0.5^{bB}
SlYABBY2b-OE	11.0±1.5^{aA}	10.0±1.5^{aA}	11.8±1.9^{aA}	12.6±5.4^{bB}	7.8±0.7^{aA}	8.0±0.7^{aA}
Type	The second inflorescence			The third inflorescence		
	Total flowers	Sepals	Petals	Total flowers	Sepals	Petals
MLK1	5.7±1.6^{aA}	9.3±0.8^{bB}	9.3±0.6^{aA}	5.7±1.0^{aA}	9.5±0.8^{bB}	9.4±0.7^{bB}
SlYABBY2b-OE	7.2±2.5^{bB}	8.6±0.5^{aA}	9.3±0.3^{aA}	5.6±0.8^{aA}	8.8±0.3^{aA}	9.1±0.8^{aA}

Values are expressed as mean±standard deviation (n=3). Values in the same column followed by different letters are statistically different between different plants: $^{a,b}P<0.05$, $^{A,B}P<0.01$. OE: overexpressing

Table 2 Phenotype of the wild-type and *SlYABBY2b* RNAi transgenic plants

Type	Plant height (cm)			Stem diameter (cm)		
	4-leaf stage	8-leaf stage	12-leaf stage	4-leaf stage	8-leaf stage	12-leaf stage
FL1	3.9±0.2^{aA}	7.7±0.4^{aA}	32.2±2.2^{aA}	0.256±0.024^{aA}	0.390±0.057^{bB}	0.839±0.068^{bB}
SlYABBY2b RNAi	4.9±0.3^{bB}	9.8±0.3^{bB}	38.6±2.8^{bB}	0.248±0.016^{aA}	0.368±0.034^{aA}	0.785±0.056^{aA}
Type	The first internodal length (cm)			The second internodal length (cm)		
	4-leaf stage	8-leaf stage	12-leaf stage	4-leaf stage	8-leaf stage	12-leaf stage
FL1	0.5±0.1^{aA}	1.0±0.2^{aA}	2.1±0.2^{aA}	0.5±0.1^{aA}	1.1±0.3^{aA}	2.0±0.1^{aA}
SlYABBY2b RNAi	0.6±0.0^{bB}	1.5±0.2^{bB}	2.5±0.2^{bB}	0.6±0.0^{bB}	1.6±0.2^{bB}	2.4±0.3^{bB}
Type	The first flower of the first truss			The second flower of the first truss		
	First fruit	Second fruit	Third fruit	First fruit	Second fruit	Third fruit
FL1	2.3±0.5^{aA}	2.1±0.3^{aA}	2.6±0.5^{aA}	2.5±0.5^{aA}	2.2±0.4^{aA}	2.4±0.5^{aA}
SlYABBY2b RNAi	2.9±0.3^{bB}	3.0±0.0^{bB}	3.1±0.3^{bB}	3.4±0.5^{bB}	3.3±0.7^{bB}	3.1±0.7^{bB}
Type	The third flower of the first truss			The first inflorescence			
	First fruit	Second fruit	Third fruit	Total flowers	Sepals	Petals	Average fruit weight (g)
FL1	2.3±0.5^{aA}	2.5±0.5^{aA}	2.2±0.4^{aA}	5.4±0.6^{aA}	5.3±0.2^{aA}	5.1±0.1^{aA}	44.5±11.7^{aA}
SlYABBY2b RNAi	3.2±0.6^{bB}	3.2±0.4^{bB}	3.1±0.7^{bB}	6.0±1.1^{bB}	5.4±0.2^{aA}	5.1±0.2^{aA}	54.4±18.4^{bB}
Type	The second inflorescence				The third inflorescence			
	Total flowers	Sepals	Petals	Average fruit weight (g)	Total flowers	Sepals	Petals	Average fruit weight (g)
FL1	5.6±0.6^{aA}	4.9±0.2^{aA}	5.0±0.0^{aA}	79.4±16.7^{aA}	5.8±0.5^{aA}	5.1±0.4^{aA}	5.1±0.2^{aA}	81.7±17.8^{aA}
SlYABBY2b RNAi	6.0±1.8^{bB}	5.1±0.2^{bB}	5.3±0.5^{bB}	87.9±23.4^{bB}	6.0±0.9^{bB}	5.6±0.4^{bB}	5.5±0.5^{bB}	82.2±7.5^{bB}

Values are expressed as mean±standard deviation (n=3). Values in the same column followed by different letters are statistically different between different plants: $^{a,b}P<0.05$, $^{A,B}P<0.01$

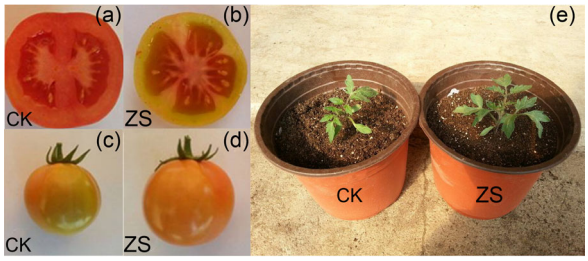

Fig. 2 Phenotypic characteristics of representative wild-type and *SlYABBY2b* RNAi transgenic plants
(a) FL1 locules. (b) *SlYABBY2b* RNAi locules. (c) FL1 fruit size. (d) *SlYABBY2b* RNAi fruit size. (e) FL1 (left) and *SlYABBY2b* RNAi plants (right) at same leaf age. CK: FL1 locules; ZS: *SlYABBY2b* RNAi locules

3.2 Expression levels of *SlYABBY2b* in transgenic tomato plants

To determine whether the difference in carpel number in our transgenic plants was caused by *SlYABBY2b*, we analyzed the expression levels of *SlYABBY2b* in the apical shoots using qRT-PCR. The expression of *SlYABBY2b* in the apical shoots of the overexpressing plants was significantly higher than that of the non-transgenic plants at every stage. In contrast, the expression of *SlYABBY2b* was significantly lower in the leaves and apical shoots of the RNAi plants than in those of the control (Fig. 3). In addition, we analyzed the expression of *SlYABBY2b* in the GA deficient mutants. In the shoot apexes of *gib* mutants (LA2893 and LA2895) and the control (LA2706), the expression of *SlYABBY2b* showed a tendency of up-down-up. The expression of *SlYABBY2b* reached its highest level during the first sampling period, and the lowest was in the second sampling period. As expected, the expression of *SlYABBY2b* in *gib* mutants was significantly higher than that in the control, and compared to LA2895, the expression of LA2893 was higher (Fig. 4).

3.3 Endogenous GA content analysis in transgenic tomato plants

The content of endogenous GA in the apical shoots of the transgenic plants during development was determined by ELISA. The endogenous GA content in the apical shoots initially decreased and then increased, whereas those in the non-transgenic plants initially increased, decreased, and finally increased. The GA content in the *SlYABBY2b*-overexpressing lines was markedly reduced compared with that in the non-transgenic lines. In contrast, the endogenous GA content in the apical shoots of

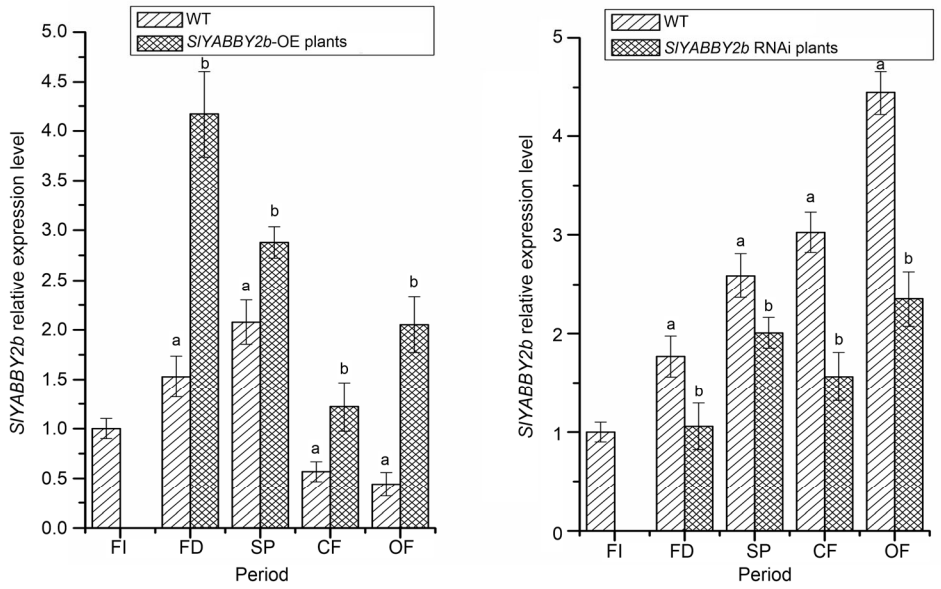

Fig. 3 qRT-PCR analysis of the expression of *SlYABBY2b* in the apical shoots of wild-type (WT) and *SlYABBY2b* transgenic plants
FI, floral bud differentiation initial stage; FD, floral bud differentiation stage; SP, sepal-petal formation stage; CF, carpel formation initial stage; OF, ovary locule complete formation stage; *SlYABBY2b*-OE, *SlYABBY2b*-overexpressing. The PCR levels were normalized to those of actin. The data represent the mean±SD of three biological samples. Values followed by the same letter (a or b) are not significantly different (P>0.05) at the same period

the RNAi transgenic plants and non-transgenic plants initially increased and then decreased. The endogenous GA content was also markedly higher in these plants than in the control (Fig. 5).

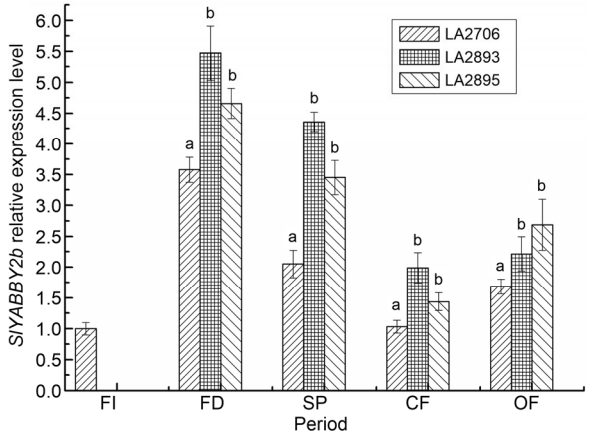

Fig. 4 qRT-PCR analysis of *SlYABBY2b* expression in the apical shoots of wild-type (WT) and *gib* mutants
FI, floral bud differentiation initial stage; FD, floral bud differentiation stage; SP, sepal-petal formation stage; CF, carpel formation initial stage; OF, ovary locule complete formation stage; LA2706, moneymaker; LA2893, *gib-1* mutants; LA2895, *gib-3* mutants. The PCR levels were normalized to those of actin. The data represent the mean± SD of three biological samples. Values followed by the same letter (a or b) are not significantly different (P>0.05) at the same period

3.4 Expression levels of GA metabolic genes in transgenic tomato plants

Changes in the GA content in the *SlYABBY2b*-overexpressing and RNAi lines may be attributed to the altered expression of those genes encoding GA metabolic enzymes. We then examined the gene expression levels of *SlCPS*, *SlGA3ox*, *SlGA20ox*, and *SlGA2ox*, in the apical shoots of the transgenic and wild-type plants at the floral bud differentiation stages using qRT-PCR. The expression levels of *SlGA20ox1* and *SlGA3ox2* were lower in the apical shoots of the *SlYABBY2b*-overexpressing plants than in those of the wild-type plants. In contrast, the transcript levels of *SlGA2ox1* and *SlGA2ox5* were higher in the transgenic plants than in the control. The expression levels of *SlGA20ox1* and *SlGA3ox2* were higher in the *SlYABBY2b* RNAi lines than in the wild-type lines, whereas the transcript levels of *SlGA2ox1* and *SlGA2ox5* were lower in *SlYABBY2b* RNAi plants than in the wild-type plants (Figs. 6 and 7).

4 Discussion

In the tomato, the shape and size of fruit are affected by the locule number. The carpels in the flower developed into locules in the fruit. *SlYABBY2b*, which encodes a *YABBY*-like transcription factor, regulates

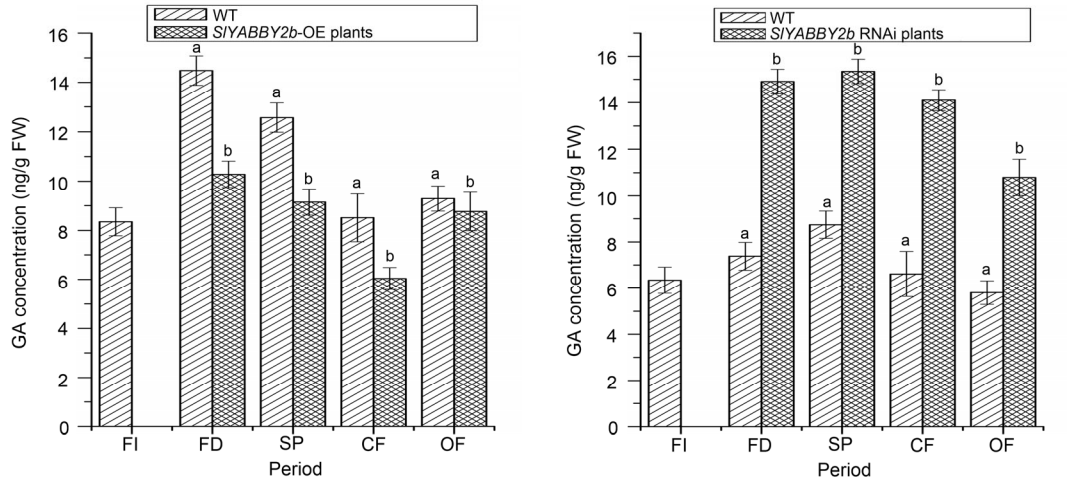

Fig. 5 Concentration of endogenous GA in the apical shoots of wild-type and *SlYABBY2b* transgenic plants
FI, floral bud differentiation initial stage; FD, floral bud differentiation stage; SP, sepal-petal formation stage; CF, carpel formation initial stage; OF, ovary locule complete formation stage; WT, wild type; *SlYABBY2b*-OE, *SlYABBY2b*-overexpressing; FW, fresh weight. The PCR levels were normalized to those of actin. The data are shown as the mean of three independent experiments. Bars indicate the standard errors (n=3). Values followed by the same letter (a or b) are not significantly different (P>0.05) at the same period

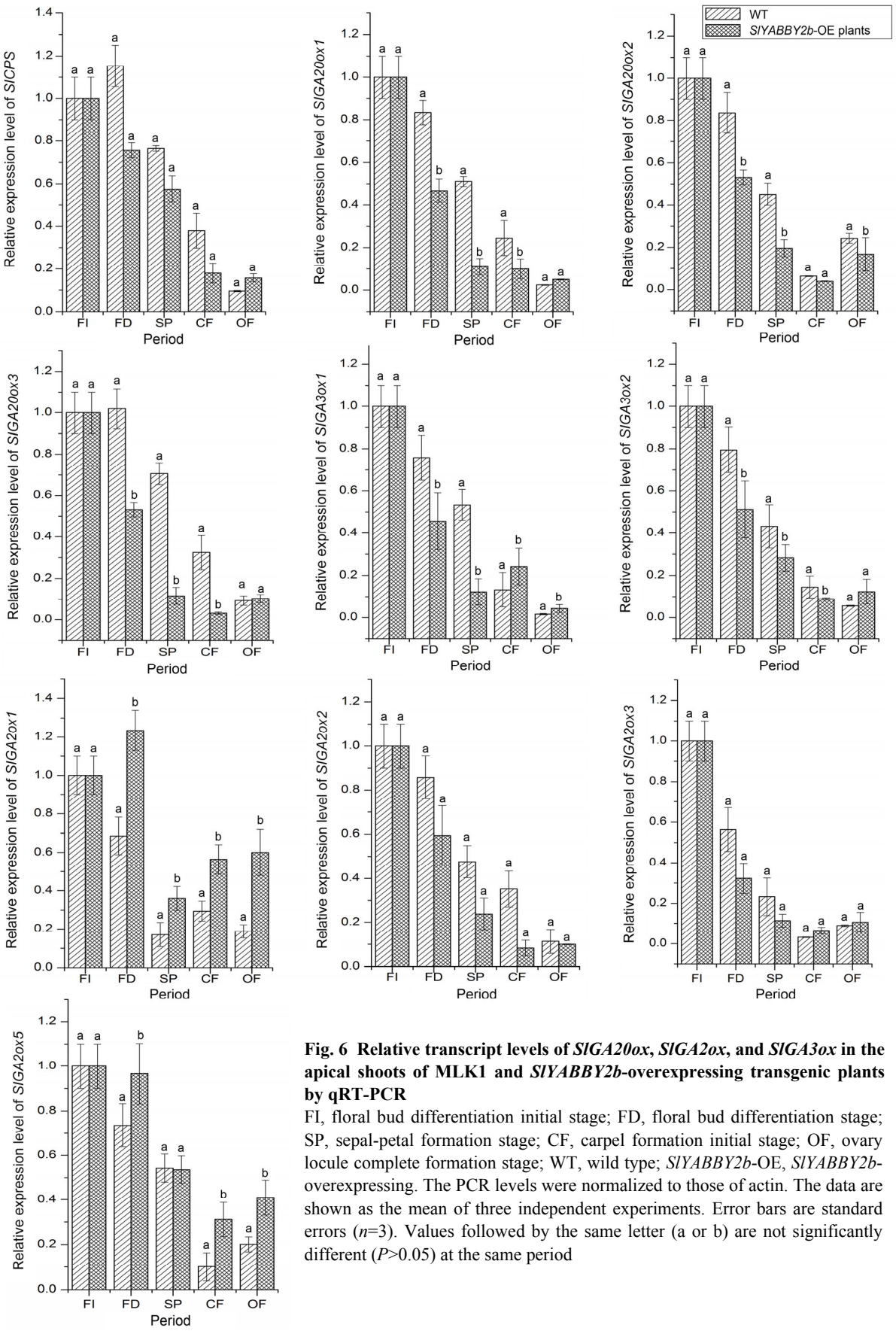

Fig. 6 Relative transcript levels of *SlGA20ox*, *SlGA2ox*, and *SlGA3ox* in the apical shoots of MLK1 and *SlYABBY2b*-overexpressing transgenic plants by qRT-PCR

FI, floral bud differentiation initial stage; FD, floral bud differentiation stage; SP, sepal-petal formation stage; CF, carpel formation initial stage; OF, ovary locule complete formation stage; WT, wild type; *SlYABBY2b*-OE, *SlYABBY2b*-overexpressing. The PCR levels were normalized to those of actin. The data are shown as the mean of three independent experiments. Error bars are standard errors (n=3). Values followed by the same letter (a or b) are not significantly different (P>0.05) at the same period

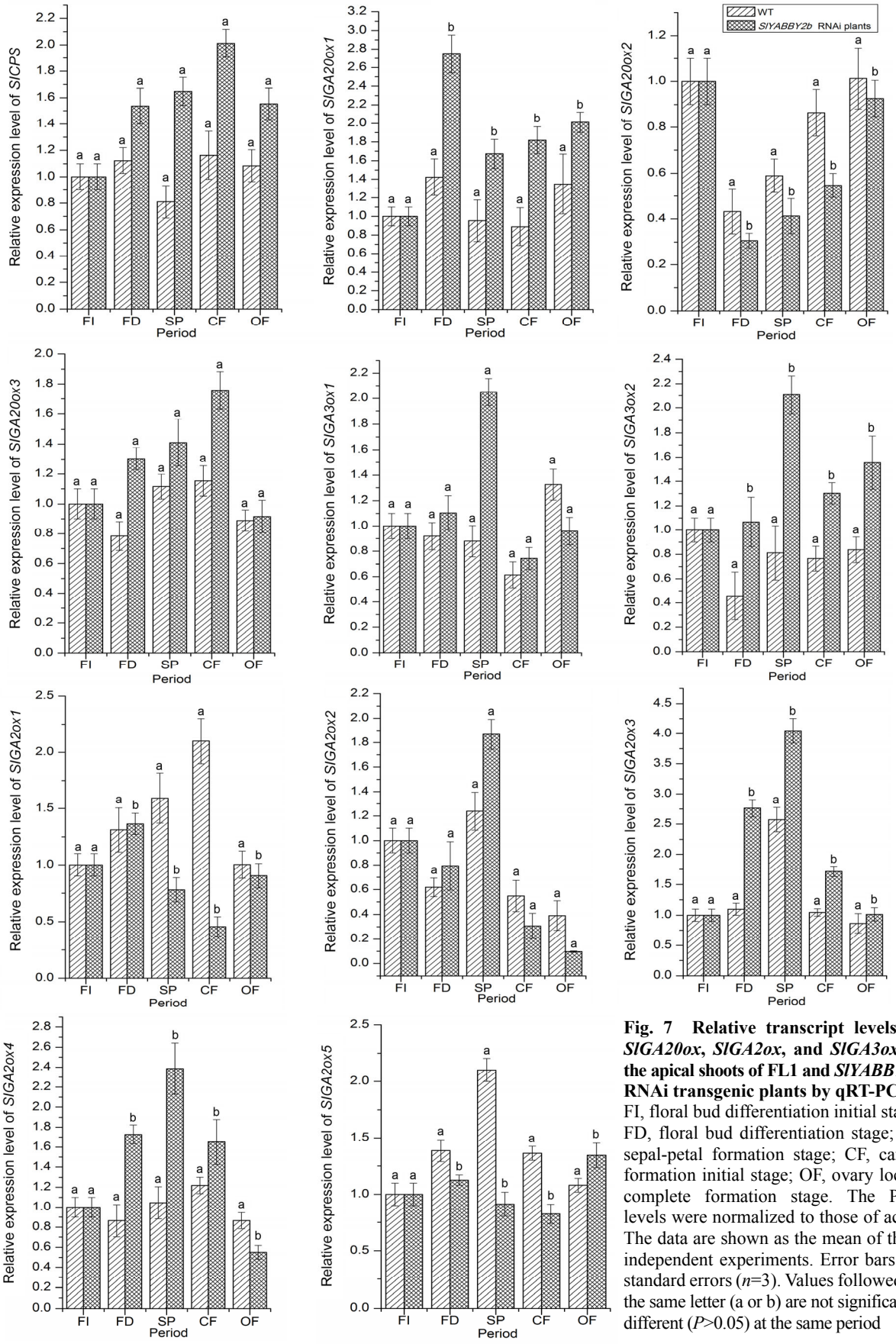

Fig. 7 Relative transcript levels of *SlGA20ox*, *SlGA2ox*, and *SlGA3ox* in the apical shoots of FL1 and *SlYABBY2b* RNAi transgenic plants by qRT-PCR

FI, floral bud differentiation initial stage; FD, floral bud differentiation stage; SP, sepal-petal formation stage; CF, carpel formation initial stage; OF, ovary locule complete formation stage. The PCR levels were normalized to those of actin. The data are shown as the mean of three independent experiments. Error bars are standard errors (n=3). Values followed by the same letter (a or b) are not significantly different (P>0.05) at the same period

fruit development by increasing locule number. High locule number is attributed to the level of *SlYABBY2b* mRNA accumulation (Cong et al., 2008). *SlYABBY2b* is a major regulator that can increase the number of locules from two to more than six (Lippman and Tanksley, 2001; Barrero and Tanksley, 2004). In the present study, we produced and characterized transgenic plants overexpressing and silencing the *SlYABBY2b* gene from tomato. Morphological alterations were observed in our transgenic plants. Overexpression of the *SlYABBY2b* gene in the multi-locule "MLK1" line decreased locule number (Figs. 1c and 1d). In contrast, RNAi silencing of the *SlYABBY2b* gene in the "FL1" line, which has few locules, increased locule number (Figs. 2a and 2b). Moreover, the plant height and internode length decreased in the *SlYABBY2b*-overexpressing plants (Fig. 1g; Table 1). In contrast, the plant height and internode length increased in the *SlYABBY2b* RNAi plants (Fig. 2e; Table 2). These results indicate that *SlYABBY2b* not only decreases the number of locules but also inhibits the growth of the tomato.

GA increases tomato ovary locule number (Sawhney and Greyson, 1971; Sawhney and Dabbs, 1978; Liu and Li, 2012). *SlYABBY2b*, which is the main regulator of tomato ovary locule number, was the first *YABBY* transcription factor found in tomato (Cong et al., 2008). *YABBY* transcription factors are closely associated with GA synthase genes in *Arabidopsis thaliana* and rice (Hay et al., 2002; Kumaran et al., 2002; Dai et al., 2007). In this study, the expression of *SlYABBY2b* was significantly higher in the apical shoots of the overexpressing plants than in those of the non-transgenic plants at every stage (Fig. 3). In addition, the endogenous GA content in the overexpressing plants was markedly lower than that in the control (Fig. 4). Conversely, the expression of *SlYABBY2b* was significantly lower in the apical shoots of the RNAi plants than in those of the wild-type plants (Fig. 3). The endogenous GA content was markedly higher in the RNAi plants than in the control (Fig. 5). Therefore, the *SlYABBY2b* gene plays a negative regulatory role in endogenous GA biosynthesis. As the first cloned *YABBY* transcription factor in tomato, *SlYABBY2b* is also involved in regulating GA biosynthesis, which is consistent with the existing findings in *A. thaliana* and rice (Hay et al., 2002; Kumaran et al., 2002; Dai et al., 2007). Furthermore, the expression of *SlYABBY2b* gene was markedly higher than that of the control in the apical shoots of GA-deficient mutants, and this showed that the GAs can inhibit the expression of *SlYABBY2b* gene negative regulation (Fig. 4).

The metabolism of GA has been comprehensively investigated (Sponsel and Hedden, 2010). These enzymes (*GA20ox*, *GA3ox*, and *GA2ox*) play a key role in controlling the bioactive GA levels (Hedden and Phillips, 2000; Sakamoto et al., 2004). The concentrations of active GA were altered in *GA20ox*, *GA3ox*, and *GA2ox* overexpressing or RNAi transgenic plants, which suggests that GA content is regulated by these genes (Hedden and Phillips, 2000). In the present study, the GA content was altered in the *SlYABBY2b*-overexpressing and RNAi lines (Fig. 4). Thus, we examined the expression levels of the genes encoding four enzymes, *SlCPS*, *SlGA3ox*, *SlGA20ox*, and *SlGA2ox* (Imai et al., 1996; Rebers et al., 1999; Serrani et al., 2007, 2008), in the apical shoots of the transgenic and wild-type plants at the floral bud differentiation stage. The present data showed that *SlYABBY2b* suppressed the expression of *SlGA20ox1* and *SlGA3ox2* but increased the expression of *SlGA2ox1* and *SlGA2ox5* in the apical shoots of the overexpressing plants (Figs. 6 and 7), thereby reducing GA content. Opposite results were found in the RNAi plants. These results indicate that *SlYABBY2b* can modulate GA synthesis. The expression levels of *SlGA20ox1*, *SlGA3ox2*, *SlGA2ox1*, and *SlGA2ox5* were significant or extremely significant, suggesting that these genes are important in the regulatory effects of *SlYABBY2b* on GA content.

In conclusion, we studied the regulatory effects of *SlYABBY2b* on tomato ovary locule number and endogenous GA content using transgenic approaches. We found that *SlYABBY2b*-overexpressing plants showed fewer locules and lower GA content than the control plants, but *SlYABBY2b* RNAi plants had more locules and higher GA content than the control plants, which suggests that the *SlYABBY2b* gene negatively regulates locule number and GA content. Furthermore GAs can also inhibit the expression of the *SlYABBY2b* gene negative regulation. In addition, the expression levels of *SlGA20ox1*, *SlGA3ox2*, *SlGA2ox1*, and *SlGA2ox5* were significant or extremely significant, suggesting that these genes are important in regulatory effects of *SlYABBY2b* on GA content,

consequently affecting GA content and tomato ovary locule number. These results suggest that the *SlYABBY2b* gene was located on GA signal transduction pathways, fed back regulation of the synthesis of GA, and felt exogenous GA signal to further regulate the formation of the tomato locule.

Compliance with ethics guidelines

Hui LI, Mei-hua SUN, Ming-fang QI, Jiao XING, Tao XU, Han-ting LIU, and Tian-lai LI declared that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 Gibberellin mutants and wild types
Table S2 RT-PCR primers used to amplify gene-specific regions

中文概要

题　目：*SlYABBY2b* 基因对番茄果实心室数和内源赤霉素含量的影响

目　的：探讨赤霉素对 *SlYABBY2b* 基因调控番茄心室形成过程中的作用，为进一步研究番茄畸形果发生机理提供了理论基础。

创新点：首次明确了 *SlYABBY2b* 基因与赤霉素的关系，且筛选出 *SlYABBY2b* 调控赤霉素合成的关键基因 *GA20ox1*、*GA3ox2*、*GA2ox1* 和 *GA2ox5*。

方　法：利用 Gateway 技术法构建 *SlYABBY2b* 基因超表达和沉默载体，并通过农杆菌介导转化法获得转基因植株。用酶联免疫吸附测定（ELISA）试剂盒检测转基因植株中赤霉素的含量，用实时荧光定量分析（qRT-PCR）赤霉素突变体中 *SlYABBY2b* 基因表达水平和 *SlYABBY2b* 转基因植株中赤霉素相关基因的转录水平。

结　论：本实验中赤霉素突变体中 *SlYABBY2b* 基因表达量显示，赤霉素短缺导致番茄植株体内 *SlYABBY2b* 基因的升高。ELISA 实验结果显示，*SlYABBY2b* 基因也能够反馈调控赤霉素的合成。qRT-PCR 结果显示，*SlYABBY2b* 基因抑制 *GA20ox1* 和 *GA3ox2* 基因的表达，促进 *GA2ox1* 和 *GA2ox5* 基因的表达。综上所述，研究结果明确了 *SlYABBY2b* 基因位于赤霉素信号转导的途径上，反馈调节赤霉素的合成，感受外源赤霉素的信号，从而进一步调控番茄心室的形成。

关键词：*SlYABBY2b*基因；赤霉素；赤霉素突变体；子房心室；转基因植株；基因表达；番茄