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ORIGINAL ARTICLE

Downregulation of *SlGRAS15* manipulates plant architecture in tomato (*Solanum lycopersicum*)

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Abstract

GRAS family transcription factors (TF) are involved in multiple biological processes in plants. In recent years among the 54 identified GRAS proteins, only few have been studied functionally in tomato (*Solanum lycopersicum*). In the present study, a novel and previously uncharacterized member of tomato GRAS transcription factors family *SlGRAS15* was isolated and functionally characterized. It was observed that *SlGRAS15* preferably expressed in roots, followed by young leaves, stem, and comparatively low transcripts levels were noticed in all other tissues. To explore the *SlGRAS15* function in detail, an RNA interference (RNAi) vector targeting *SlGRAS15* was constructed and transformed into tomato plants. The transgenic plants carrying *SlGRAS15*-RNAi displayed pleiotropic phenotypes associated with multiple agronomical traits including reduced plant height and small leaf size with pointed margins, increased node number, lateral shoots, and petiolules length. In addition, transcriptional analysis revealed that silencing *SlGRAS15* altered vegetative growth by downregulating gibberellin (GA) biosynthesis genes and stimulating the GA deactivating genes, thus lowering the endogenous GA content in tomato transgenic lines. Moreover, the GA signaling downstream gene (*SlGAST1*) was downregulated but the negative regulator of GA signaling (*SlDELLA)* was upregulated by *SlGRAS15* silencing. The root and hypocotyl length in *SlGRAS15*-RNAi lines showed reduced growth under normal conditions (Mock) as compared with the wild type (WT) control plants. Taken together, these findings enhanced our understanding that suppression of *SlGRAS15* lead to a series of developmental processes by modulating gibberellin signaling and demonstrate an association between the *SlGRAS15* and GA signaling pathway during vegetative growth in tomato.

Keywords Tomato · *SlGRAS15* · Downregulation · Plant architecture · Gibberellin

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Introduction

GRAS proteins are plant-specific transcription factors that play critical roles in plant development and signal transduction pathways, including gibberellin biosynthesis and signaling (Lee et al. 2002), auxin signaling (Gao et al. 2004; Sánchez et al. 2007), phytochrome signaling (Torres-Galea et al. 2006), lateral shoot formation (Li et al. 2003), and gametogenesis (Morohashi et al. 2003). Typically, members of the GRAS (named after GAI, RGA, SCR) family show high sequence similarity to each other in their C-terminus, where motifs comprising of LHRI, LHRII, PFYRE, VHIID, and SAW can be seen (Bolle 2004; Pysh et al. 1999). In distinction, N-terminus of GRAS TF differs in sequence and length, which appears like the foremost contributor to the specific function of every gene (Sun et al. 2011; Tian et al. 2004).

SlGRAS15 belongs to SCR subfamily and is more closely correlated to AtSCR in *Arabidopsis*, StSCR in *Solanum tuberosum*, and CaSCRL in *Capsicum annum* than any other

protein, but the function of these three proteins except SlGRAS15 is well known (Cui et al. 2007). Tomato GRAS proteins genome wide analysis has identified 54 GRAS proteins (Niu et al. 2017). However, only eight GRAS proteins that have been functionally characterized, which comprise of SlLS, SlDELLA, SlGRAS2, SlGRAS6, SlGRAS26, SlGRAS24, SlGRAS38, SlGRAS7, and SlGRAS40. Upregulation of two homologous genes *SlGRAS24* and *SlGRAS40*, belongs to HAM subfamily in *Arabidopsis* developed same phenotypes, such as dwarf plant height, delay in flowering time, fewer number of flowers, lower seed number, and lower percentage of fruit set (Huang et al. 2017; Liu et al. 2017). The *SlFSR* (*SlGRAS38*) controls fruit shelf life by altering the fruit cell wall metabolism (Zhang et al. 2018), while downregulation of *SlGRAS26* play a vital role in the initiation of lateral and inflorescence meristems in tomato (Zhou et al. 2018).

As an important growth regulator, gibberellin (GA) plays an essential role in plant growth and development processes including endosperm mobilization, seed germination, stem elongation, root development, leaf expansion, flowering, pollen maturation, and fruit set (Claeys et al. 2014; Hedden and Phillips 2000). Recently, many studies contribute to the understanding of GA biosynthesis. For instance, severe GA biosynthesis defective mutants (such as the deletion of the *GA1* gene in *ga1-3*) show defects in development, and gibberellin deficient dwarf phenotypes are restored to WT by applying exogenous gibberellic acid (GA_3) (Magome et al. 2004; Olimpieri et al. 2011). In comparison, mutants impaired in gibberellin signaling present similar phenotypes but these are not rescued by exogenous GA_3 (Harberd et al. 2009).

The function of GRAS genes has been well studied in various plants species, it is essential to study the role of all other GRAS proteins in tomato. The *GRAS24* gene is involved in downstream GA signaling (Huang et al. 2016), while *GRAS2* function in the upstream GA biosynthesis (Li et al. 2018). This different role of *GRAS24* and *GRAS2* in GA signaling pathway further confirms the importance of GRAS gene in GA stability. The numerous functions of gibberellin are a result of the periodic and spatial regulation of gibberellin biosynthesis, perception, and subsequent signal transduction (Olszewski et al. 2002). *AtSCL3* acts as an integrator of *DELLAs* and SHR-SCR, a complex in which two types of GRAS proteins mediates GA dependent elongation of cells in the root endodermis. The SHR-SCR complex combined with auxin influx carriers *LAX3* and *AUX1* has synergetic effects on primary/lateral root development in *Arabidopsis* (Della Rovere et al. 2015).

Tomato is a very important crop for human diet and model species due to its significance as the availability of molecular tool and efficient transformation. *Arabidopsis* sleepy1 (*sly1*) and potato photoperiod responsive 1 (*phor1*) are some GA-deficient mutants, which are insensitive to exogenous GA application due to the deficiency in the GA signal transduction pathway (Amador et al. 2001; McGinnis et al. 2003). Though the rest of GA-deficient mutants responsible for the inhibition of GA biosynthesis pathway, such as semi dwarf 1 (*sd1*), can be restored by the GA application (Neeraja et al. 2009). In tomato, a putative DELLA mutant procera (pro) displays a well-characterized constitutive GA response phenotype (Bassel et al. 2008) and homeostatic GA responses are regulated by both DELLA-dependent and independent pathways (Livne et al. 2015). Identifying the role of other GRAS family genes in crops like tomato would help to clarify the molecular mechanisms regulating developmental process and possibly assist the breeding of efficient varieties.

Here, we report the function of *SlGRAS15* (Solyc10g074680), a member of SCR subfamily and aimed to discover more about its function in tomato plant development. The analysis of expression profile showed that *SlGRAS15* is highly expressed in young leaves, stem, and root. Moreover, the expression level of *SlGRAS15* can be increased by external application of GA, ABA, and IAA. The promoter of *SlGRAS15* was analyzed and found to be associated with both gibberellin- and auxin-related cis-acting elements. To further apprehend the role of *SlGRAS15* in tomato growth and development, we prepared a specific RNAi vector to generate *SlGRAS15*-RNAi lines, and pleiotropic phenotypes associated with multiple agronomical traits were observed. The short height of plants was rescued by exogenous application of GA_3 further supporting the observation that *SlGRAS15* transgenic lines are GA sensitive. Phenotypic and gene expression analyses are reported here to disclose the probable bases of all these phenotypes, which would help to increase our knowledge of GRAS proteins in the regulation of plant growth and developmental processes.

Materials and methods

Plant materials and growth conditions

In this study the WT tomato (*Solanum lycopersicum* Mill. cv. Ailsa Craig AC^{++}) and transgenic lines were grown in a controlled greenhouse standard condition (25 °C/18 °C temperature day/night, day night cycle 16 h/8 h, light intensity of 250 μmol m^{-2} s^{-1}, and humidity 80%) managed and watered regularly. For experiments of tissue culture, seeds of tomato were surface sterilized using sodium hypochlorite solution, then immersed and shaked in sterilized distilled water up to 3 days to germinate and sowed on 1/2 MS solid agar medium (pH ¼ 5.8). Transgenic tomato lines of the first generation (T_0) came from tissue culture, second generation (T_1), and third generation (T_2) from seeds respectively, were used in this study.

For tissues/organs specific expression profiling of *SlGRAS15* gene, roots, stems, young leaves, mature leaves,

senescent leaves, flowers (tagged at anthesis), sepals, and tomato fruits of five developmental stages were collected. The fruit at following development stages were collected; IMG (immature green at 20 days post anthesis (DPA), MG (mature green at 35 DPA), B (breaker with the start of change in color from green to yellow), B4 (4 days after breaker stage), and B7 (7 days after breaker) (Xie et al. 2014). The fifth leaf from the top and young stem of the consistent developmental stage of 90 days old plants were collected and used for analysis of gene expressions. All the tissues were gathered in the start of July, flowers were collected early in the morning while rest of the tissues were collected at noon, frozen in liquid nitrogen immediately and stored at − 80 °C until required.

Sequence, structure, and phylogenetic analysis

The cDNA and protein data of *SlGRAS15* gene were retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) and Sol Genomics Network (SGN) (http://solgenomics.net/). The DNAMAN 6.0 software was used to carry out multiple sequence alignments of SlGRAS15 with other proteins, while Scan-Prosite (http://prosite.expasy.org/scanprosite/) was used for the annotation of conserved functional domains. For phylogenetic study, the MEGA 6.06 software (http://megasoftware.net/) was used. A total of 18 GRAS peptide sequences were used to construct an unrooted neighbor-joining phylogenetic tree with the bootstrap set at 1000 replicates. GenBank and Sol Genome Network genes accession numbers for multiple sequence alignment and phylogenetic study are as follow: SlGRAS15 (Solyc10g074680), StSCR (XM_006365258.2), CaSCRL (XM_016690185.1), NtSCRL (XM_016630625.1), AtSCR (NM_115282.4), BnSCR-like (XM_022708083.1), GmSCR (XM_003551583.4), OsSCR1-like (XM_015759916.2), SlGRAS43 (Solyc09g066450.1), SlGRAS38 (Solyc08g080400.1.1), AtSCL4 (Solyc02g085340.1.1), SlGRAS2 (Solyc07g063940.1.1), AtPAT1 (Solyc11g012510.1.1), SlLS (Solyc07g066250.1.1), SlDELLA (Solyc11g011260.1.1), ZmGA1-like (NM_001319732.2), SlGRAS24 (Solyc01g090950.2.1), SlGRAS37 (Solyc08g078800.1.1), and SlGRAS40 (Solyc08g078800.1). For the analysis of putative cis-elements in the promoter sequence of *SlGRAS15* (2000 bp at 5′ upstream end) were predicted against the promoter database PLACE (http://www.dna.affrc.go.jp/PLACE/signalscan.html) (Higo et al. 1999) (Table S2).

Plasmid construction and plant transformation

For *SlGRAS15*-RNAi plasmid construction, the pBIN19 vector was used. A 429-bp-long fragment of *SlGRAS15* DNA at the mRNA 5′-end was used in the hairpin and amplified by using the *SlGRAS15*-F/R specific primers (Table S1). The construct was transformed into tomato cv. (AC^{++}) Ailsa Craig by *Agrobacterium tumefaciens* (strain LBA4404) (Chen et al. 2004). MS medium containing kanamycin was used to screen positive transformed lines, which were analyzed by genomic PCR to determine the existence of T-DNA using the primers *NPTII*-F/R (Table S1).

Plant treatments

The tomato seedlings at five leaf stage were used for treatments and each treatment is repeated three times. WT seedlings of tomato were sprayed with IAA 50 μM, GA_3 50 μM, ABA 100 μM, or distilled water used as control (mock). After treatment the third leaves of the seedlings at 0, 1, 2, 4, 8, 12, and 24 h were harvested, instantly kept in liquid nitrogen and stored at − 80 °C. The dwarf transgenic plants were sprayed with 100 μM GA_3 on every 3rd day up to 2 weeks, starting from 30 days after sowing while the non-treated plants were used as control.

Quantitative real-time PCR analysis

The total RNA was extracted from various tissues of WT and *SlGRAS15*-RNAi lines (T_2) by using RNAiso Plus (Takara) following manufacturer's instructions. For reverse transcription reaction we used 1 μg of RNA and the M-MLV reverse transcriptase kit (Promega). The GoTaq qPCR Master Mix (Promega) and the CFX96™ Real-Time System (Bio-Rad) were used for qRT-PCR analysis. An internal reference gene the Clathrin Adaptor Complex, *SlCAC* (accession number: SGN-U314153) (Expósito-Rodríguez et al. 2008; González-Aguilera et al. 2016) was used in this study for data normalization. All the experiments were performed in triplicate. The specific primers of every gene being used for qRT-PCR are detailed in Table S1.

Measurement of plant architecture parameters

To relate the morphological variances of RNAi lines and WT, several parameters, such as height of plant, length, and width of leaf, leaf shape index (length to width ratio), internode length, petiolules length, and branches number (≥ 0.5 cm) were analyzed. Moreover, height of the plant was measured at the 20th day after germination and once each week up to 2 months. For all parameters of RNAi lines and WT, a minimum of ten plants were used from each line.

Histological observation

Leaf and mature apical leaflets from the similar position under the first inflorescence of WT and *SlGRAS15*-silenced lines were harvested and dissected. For root microscopy, transverse section of primary root from WT and *SlGRAS15*-RNAi lines

were selected. The harvested samples were instantly fixed by 70% FAA (ethanol/acetic acid/formaldehyde 18:1:1) followed by dehydration, fixation, sectioning, dewaxing, and staining with safranin and fast green. Then analyzed under a light microscope (OLYMPUS IX71, Japan) and photographs were captured.

GA_3 contents quantification

Mature leaves of WT and *SlGRAS15*-RNAi lines were collected and frozen immediately in liquid nitrogen for GA_3 content determination. For the extraction and purification of gibberellin (GA_3) gibberellin (GA_3) kit (GA-4-Y Comin Biotechnology Co., Ltd., China) was used following manufacturer's guidelines. The High Performance Liquid Chromatography (HPLC) analyses were performed to measure the total volume of biologically active endogenous gibberellin (GA_3) in the mature leaves of WT and *SlGRAS15*-RNAi lines.

Statistical analysis

The mean values of data were evaluated from three replicates and standard error (SE) of the means was calculated. Statistical analysis of data was performed using Sigmaplot 12.1. (SYSTAT and MYSTAT Products, USA, and Canada) and *t* test (SAS 9.2) was used for measuring significant differences between the means.

Results

SlGRAS15 is a member of SCR subfamily in tomato

To explore the potential function of *SlGRAS15* gene in tomato, we isolated a putative *SlGRAS15* gene from WT tomato fruits based on a cDNA clone. The analysis of nucleotide sequence showed that *SlGRAS15* was consistent with the cDNA clone, contained an ORF (open reading frame) of 2481-bp-long coding an 827 amino acid putative protein having a typical region of GRAS domain at their C-terminus. To examine the GRAS domain of *SlGRAS15* gene in more detail, the full length amino acid sequence of 18 proteins including SlGRAS15, StSCR, CaSCRL, NtSCRL, AtSCR, BnSCR-like, GmSCR, OsSCR1-like, SlGRAS43, SlGRAS38, AtSCL4, SlGRAS2, AtPAT1, SlLS, SlDELLA, ZmGA1-like, SlGRAS24, SlGRAS37, and SlGRAS40 were considered for multiple sequence alignments (Fig. S1). The results showed five conserved motifs are present including LHRI, LHRII, VHIID, PFYRE, and SAW at their C-terminus. Though, these proteins vary in sequence and length at N-terminus. To study phylogenetic relationships among GRAS proteins in *Arabidopsis*, tomato, and other plant species, we used the amino acid sequences to construct an unrooted neighbor-joining phylogenetic tree (Fig. 1a). From the analysis of the phylogenetic tree, our newly identified *SlGRAS15* protein belonged to the growth-related SCR subfamily of GRAS proteins according to their relative functions. Additionally, the results also showed that most of the SCR

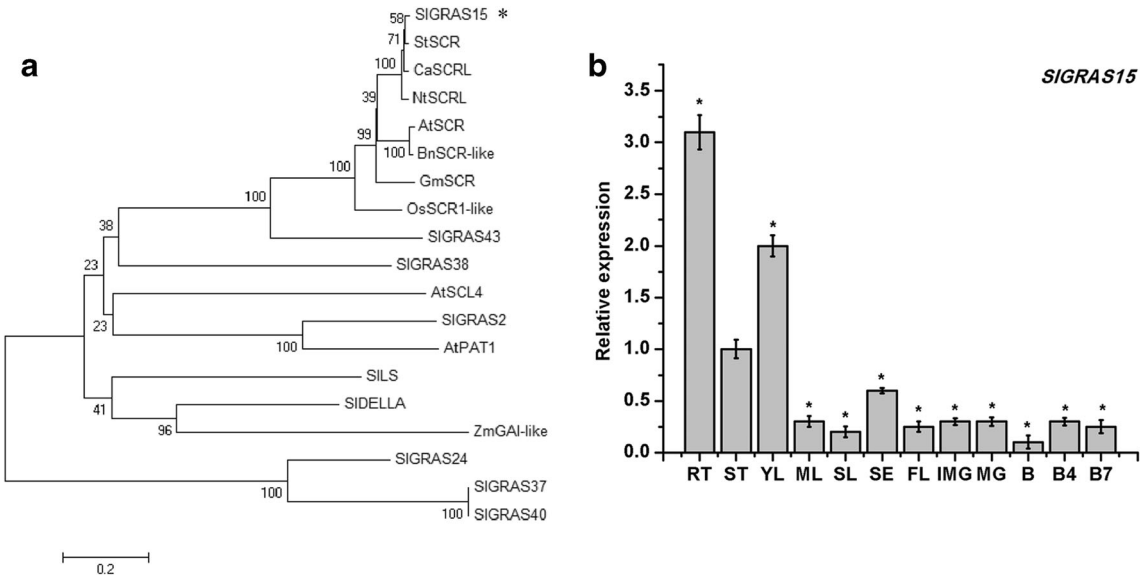

Fig. 1 Phylogenetic analysis and relative endogenous expression of *SlGRAS15* in various tissues at different development stages in wild type (WT) tomato plants. **a** Phylogenetic analysis of *SlGRAS15* with other proteins was constructed by the neighbor-joining approach by MEGA 6.06. Values above the branches are bootstrap percentages analysis of 1000 replicates. *Sl* (*Solanum lycopersicum*), *St* (*Solanum tuberosum*), *Ca* (*Capsicum annuum*), *Nt* (*Nicotiana tabacum*), *At* (*Arabidopsis thaliana*), *Bn* (*Brassica napus*), *Gm* (*Glycine max*), *Os* (*Oryza sativa*), and *Zm* (*Zea mays*). **b** The expression pattern of *SlGRAS15* in WT tomato. RT, root; ST, stem; YL, young leaf; ML, mature leaf; SL, senescent leaf; SE, sepal of flower in anthesis; F, flower; IMG, immature green fruit; MG, mature green fruit; B, breaker fruit; B4, 4 days after breaker fruit; B7, 7 days after breaker fruit. The relative expression levels in ST were set to 1. Each value represents the mean ± SD of three independent biological replicates ($n = 3$). Asterisks indicate a significant difference ($P < 0.05$) between ST and other tissues

proteins with similar functions could be classified into the same subgroups on the basis of similarities in their GRAS domains, thus indicating that it may also have a crucial role in modulating gibberellin signaling.

Expression pattern analysis of *SlGRAS15* gene in different tomato tissues

To further understand the role of *SlGRAS15* gene in tomato growth and development, the transcript accumulation of *SlGRAS15* gene was analyzed by qRT-PCR in different plant parts, including vegetative tissues such as young leaves (YL), mature leaves (ML), senescent leaves (SL), stems (ST), roots (RT), and reproductive tissues including sepals (SE), flowers (FL), and fruits at different developmental stages such as immature green (IMG), mature green (MG), Breaker (B), 4 days after breaker (B4), and 7 days after breaker (B7) (Fig. 1b). The results revealed that *SlGRAS15* gene shows high expression in roots, followed by young leaves, stem, and comparatively low transcripts levels were detected in all remaining tissues, which reveals the tissue specific expression in tomato. This gradual ascending expression of *SlGRAS15* in tomato indicates that *SlGRAS15* may contribute in the development of roots, leaf, and stem but not in developing fruits.

SlGRAS15 contributes to morphogenesis during vegetative growth in tomato

To ascertain the role of *SlGRAS15* in tomato, a 429-bp fragment of *SlGRAS15* near the N-terminal was cloned to suppress the endogenous expression levels of *SlGRAS15* through RNAi silencing. Nine transgenic independent RNAi lines were obtained. The silencing efficiency of *SlGRAS15* transgenic line 1, 3, and 4 were 79%, 85%, and 95% respectively, and were labeled as RNAi1, RNAi3, and RNAi4 (Fig. 2a) and were selected for further study. Under the same growth conditions these RNAi lines showed the same phenotypes in vegetative growth and development when observed visually. The height and length of first four internodes were measured. We observed that *SlGRAS15*-RNAi lines showed a reduction in plant height compared with the WT plants (Fig. 2b–d).

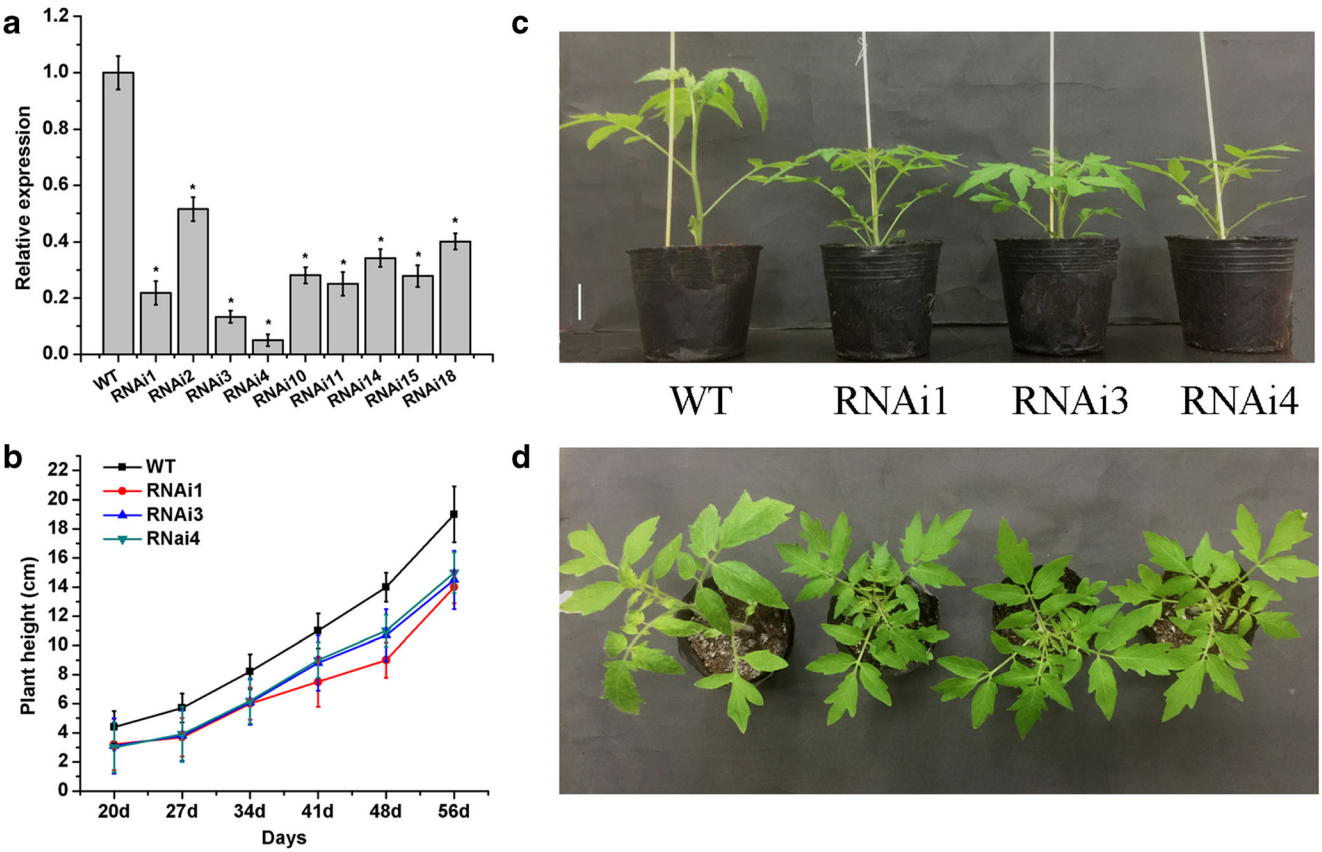

Fig. 2 Phenotypes and expression analysis of *SlGRAS15* in wild type (WT) and transgenic lines. **a** The expression levels of *SlGRAS15* in transgenic and WT plants, examined by real-time qRT-PCR, using young leaves of the plants as tissue sample. The expression data of WT plants were normalized to 1. **b** The growth rate of WT and *SlGRAS15*-RNAi lines. The plants height was measured every 7 days starting from 20 days to 2 months. **c** The *SlGRAS15*-RNAi plants (RNAi1, RNAi3, and RNAi4) were dwarfed; the picture was taken at 2 months after colonization. **d** Vertical view of WT and transgenic plants. Each value represents the mean ± SD of three independent biological replicates ($n = 3$). Bar = 1 cm. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines

The number of nodes and mature compound leaves number were significantly increased while length and width of mature leaflets decreased in *SlGRAS15*-RNAi lines as compared with the WT. However, the leaf shape indexes remained unchanged. The length of petiolules in *SlGRAS15*-RNAi lines were all longer than WT plants, while the 4 internodes length in *SlGRAS15*-RNAi line were smaller than WT plants. This demonstrates that the dwarf transgenic lines depicted the shortening of internodal length (Fig. 3a–h). Besides, in *SlGRAS15*-RNAi lines mature leaves were smaller in size than WT, we also observed that the margins of leaflets of transgenic lines were pointed (Fig. 3a). Two genes *GOB* (*Goblet*) (Berger et al. 2009) and *NAM2* (Hendelman et al. 2013) of NAC TF family are considered to be involved in the maintenance and establishment of leaf margins. Therefore, we measured the expression of these two genes and significant increase in the expression levels of these genes were observed in RNAi lines than in WT (Fig. S2). Furthermore, microscopic analyses of the transverse section of leaf of RNAi lines displayed reduced leaf thickness, which can be attributed to the spongy mesophyll that seems thicker in the WT compared with the RNAi line. The spongy mesophyll of RNAi line contain less cell layers and are more disorganized than in the WT (Fig. 4a, b). In addition, microscopic study of roots showed that *SlGRAS15*-RNAi lines also exhibited less cell layers and more disorganized structure as compared with WT plants (Fig. 4c).

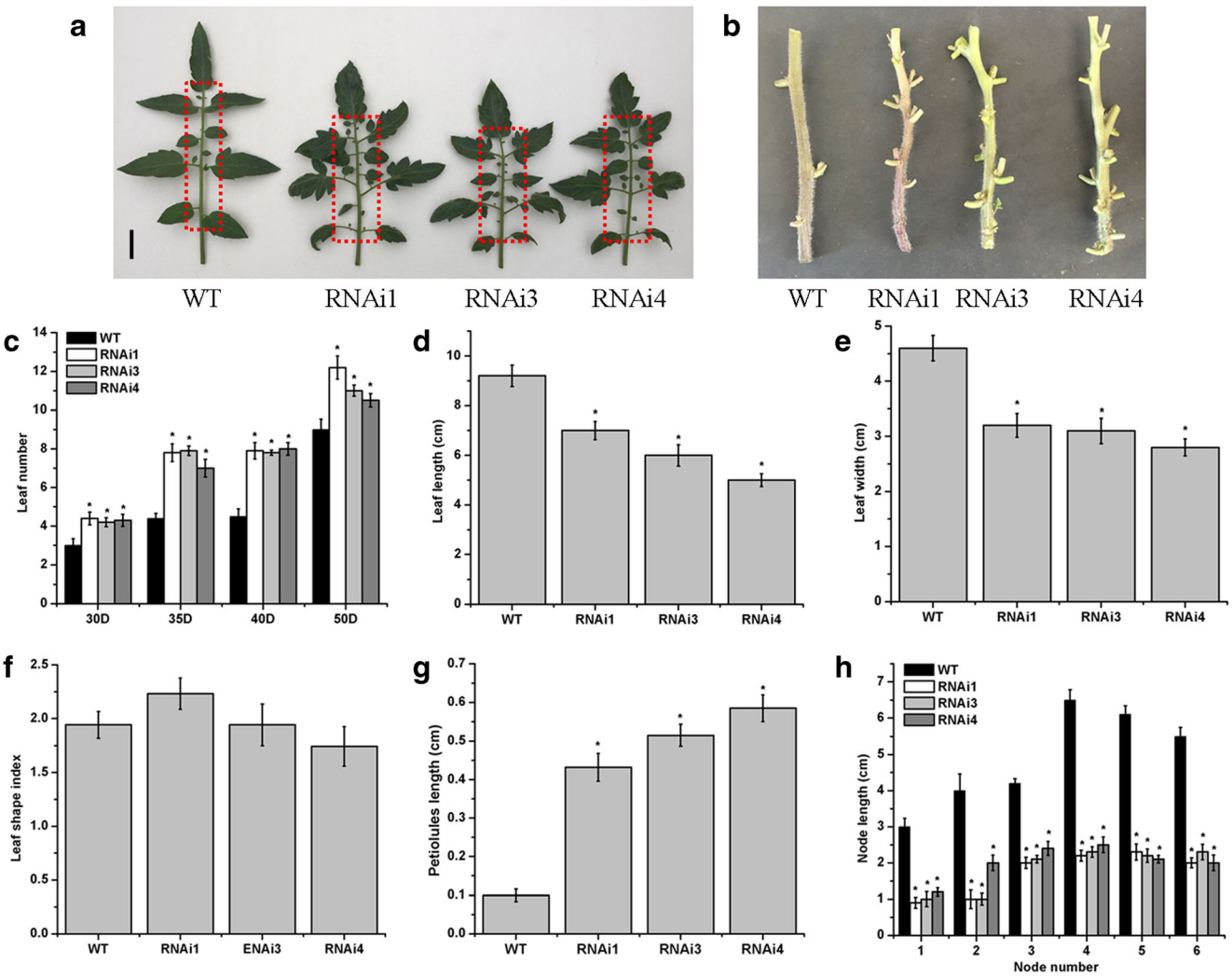

Fig. 3 Phenotypes of wild type (WT) and *SlGRAS15*-silenced lines. **a** Phenotypes of leaf number, petiolules length, and leaf size of WT and *SlGRAS15*-RNAi lines. **b** Node number of WT and *SlGRAS15*-RNAi lines. **c** Number of leaves starting from 30 to 50 days of *SlGRAS15*-RNAi lines and WT plants. **d** Leaf lenght. **e** Leaf width of WT and *SlGRAS15*-silenced lines. The fourth leaf from the top of 2-months-old transgenic and non-transgenic plants was used for leaf size measurement. **f** Leaf shape index of WT and *SlGRAS15*-RNAi lines. **g** Length of petiolules from *SlGRAS15*-RNAi lines and WT plants. **h** Node length of *SlGRAS15*-RNAi lines and WT plants. Each value represents the mean ± SD of three independent biological replicates ($n = 3$). Bar = 1 cm. Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines

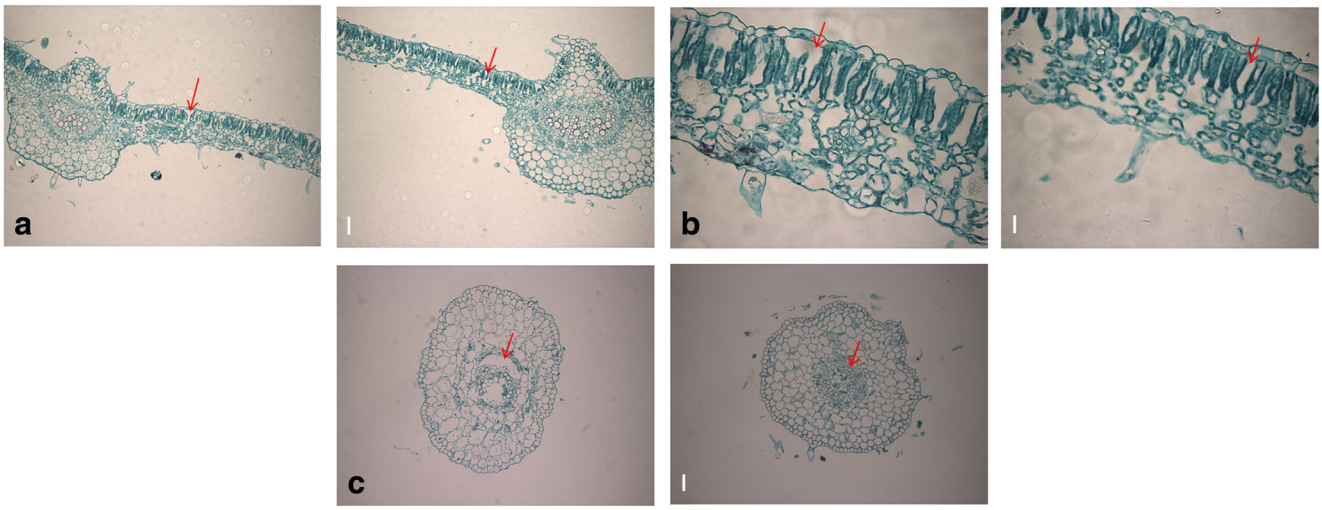

Fig. 4 Microscopy of leaf and roots from wild type (WT) and *SlGRAS15*-RNAi lines. **a** Transverse section of leaf from WT (left) and RNAi transgenic line (right); bars = 9 μm. **b** Cell layers from WT (left) and RNAi transgenic line (right); bars = 9 μm. The samples were prepared from 60-days-old plants. **c** Transverse section of root from WT (left) and RNAi transgenic line (right). The samples were prepared from 8-days-old seedlings

Effect of various phytohormones on expression of *SlGRAS15* gene

The plant growth hormones play vital roles in various environmental signals and developmental processes. Therefore, the transcript accumulation level of *SlGRAS15* under various hormones treatments was analyzed by qRT-PCR. The qRT-PCR analysis revealed that the transcript accumulation of *SlGRAS15* was significantly increased by exposure to ABA in all treatments except at 2 h, but exhibiting a peak at 24 h; IAA induced *SlGRAS15* expression significantly in all treatments but peaked at 12 h. Moreover, the GA_3 treatment reduced *SlGRAS15* expression in all treatments except at 12 h and 24 h where its transcript accumulation was significantly induced (Fig. 5a–c). The transcript accumulation of *SlGRAS15* was reduced by ACC (ethylene precursor) but showed significant increase only at 12 h stage. Indicating that downregulation of *SlGRAS15* weakens the responsiveness to ethylene. In addition, the MeJA reduced *SlGRAS15* expression in all treatments except at 2 h and 8 h where its transcript accumulation was significantly induced (Fig. 5d, e). These findings suggest that *SlGRAS15* may be involved in phytohormones biosynthesis or signaling.

To further understand the sensitivity of *SlGRAS15*-RNAi lines to plant growth hormones, exogenous ABA, IAA, GA_3, ACC, and MeJA were applied to WT and *SlGRAS15*-RNAi seedlings to investigate whether downregulation of *SlGRAS15* altered other aspects of ABA, IAA, GA_3, ACC, and MeJA dependent plant growth. The *SlGRAS15*-RNAi lines showed significant reduction in hypocotyl length except the RNAi1 line which showed non-significant reduction in hypocotyl length under normal conditions (Mock) as compared with the WT control plants. While the root length in *SlGRAS15*-RNAi lines were non-significantly reduced under normal conditions (Mock) compared with the WT (Fig. S3). In the presence of 2 and 5 μM ABA treatment, hypocotyl and root growth was inhibited in *SlGRAS15*-RNAi seedlings than the WT plants. The exposure of *SlGRAS15*-RNAi seedlings to 5 and 10 μM IAA caused slightly short hypocotyl and root length than WT seedlings, indicating that seedlings of *SlGRAS15*-RNAi lines were somehow sensitive to auxin when *SlGRAS15* was downregulated. Similarly, *SlGRAS15*-RNAi lines were grown on MS media supplemented with 2 and 5 μM GA_3 inhibited the hypocotyl and root growth as compared with the WT, reducing the initial difference in length (Fig. S3). These results suggest that *SlGRAS15* is involved in ABA, IAA, and GA_3, dependent pathways to regulate plant growth and development. Moreover, the transcript accumulation of *SlGRAS15* was in part regulated by ABA, IAA, and GA_3. Analysis of promoter sequence confirmed that there are various cis-elements related to plant hormones present in the promoter region of *SlGRAS15* gene, including ABA, IAA, GA_3, MeJA, and Ethylene responsive (Table S2). The presence of these cis-elements showed that they have possible and important function in phytohormones response (Fig. 5). Our study emphasized that the *SlGRAS15* expression is hormone inducible and produce a signaling cascade during growth and development in tomato plants.

SlGRAS15-RNAi lines exhibits increased lateral shoot number in tomato

Besides the dwarf plant and small leaf, we also observed that the number of lateral shoots in *SlGRAS15*-RNAi lines was much higher than that of WT, weaker apical dominance and reduced plant height may also be attributed to the higher lateral shoots number. To find out the possible

reason for this phenotype of RNAi lines, we studied the expression level of two genes *Blind* (*SlBL*) (Schmitz et al. 2002) of class R2R3 from Myb TF family act as a positive regulator in the development of lateral meristems and *BRC1b*/*TCP8* (Martin-Trillo et al. 2011) related to the development of axillary shoots, in WT and *SlGRAS15*-RNAi lines. The *SlBL* gene expression level is induced in *SlGRAS15*-RNAi lines while the *SlBRC1b*, a TCP transcription factor family member controls cell proliferation and elongation in the lateral organs and meristems of tomato (Martin-Trillo et al. 2011) were significantly downregulated in transgenic lines (Fig. S2).

Silencing *SlGRAS15* regulates the transcripts accumulation of gibberellins-related genes

To find out the effect of *SlGRAS15* silencing on gibberellin metabolism, the expression level of GA biosynthetic genes in RNAi lines and WT stems were determined. Two genes *CPS* and *KAO* in the GA biosynthetic pathway involved in the preliminary steps of GA biosynthesis were significantly downregulated in the *SlGRAS15*-RNAi lines (Fig. 6a, b). Furthermore, *GA3ox*s are able to catalyze the last step to crop bioactive endogenous GAs (GA_1, GA_3, and GA_4) (Hedden and Kamiya 1997; Yamaguchi 2008). In *SlGRAS15* silenced lines, the transcript accumulation of *GA3ox1* and *-2* was significantly reduced, indicating that the GA biosynthetic pathway was inhibited in the transgenic plants (Fig. 6c, d). The expression level of *SlGA2ox1*, and *-2* coding for *GA2ox*s (the central GA catabolic enzyme) were assessed by qRT-PCR. These two genes were induced in the *SlGRAS15*-RNAi lines as compared with WT plants (Fig. 6e, f). This revealed that downregulation of *SlGRAS15* might halt the GA biosynthesis pathway but stimulates the pathway involved in GA inactivation resulting in GA deficiency. However, the transcript level of *DELLA* protein, a negative regulator of gibberellin signaling pathway, was increased in RNAi lines (Fig. 6g). Expression levels of tomato gibberellin stimulated transcripts1 (*SlGAST1*); a GA responsive downstream gene (Ding et al. 2013) in tomato was significantly suppressed in RNAi lines (Fig. 6h). This showed that downregulation of *SlGRAS15* alters GA biosynthesis, signal transduction, and its catabolism.

Bioactive endogenous GA_3 contents in WT and *SlGRAS15*-RNAi lines were also measured, the results confirmed that GA_3 contents was decreased in *SlGRAS15*-RNAi lines than WT (Fig. 7a), indicating that the dwarf phenotypes may be due to reduced endogenous bioactive GA_3. To further confirm this notion, exogenous application of 100 μM GA_3 on every 3rd day for 2 weeks were adopted to recover the dwarf plant phenotypes of *SlGRAS15*-RNAi lines and confirmed that RNAi plant were able to restore their height to WT levels (Fig. 7b, c).

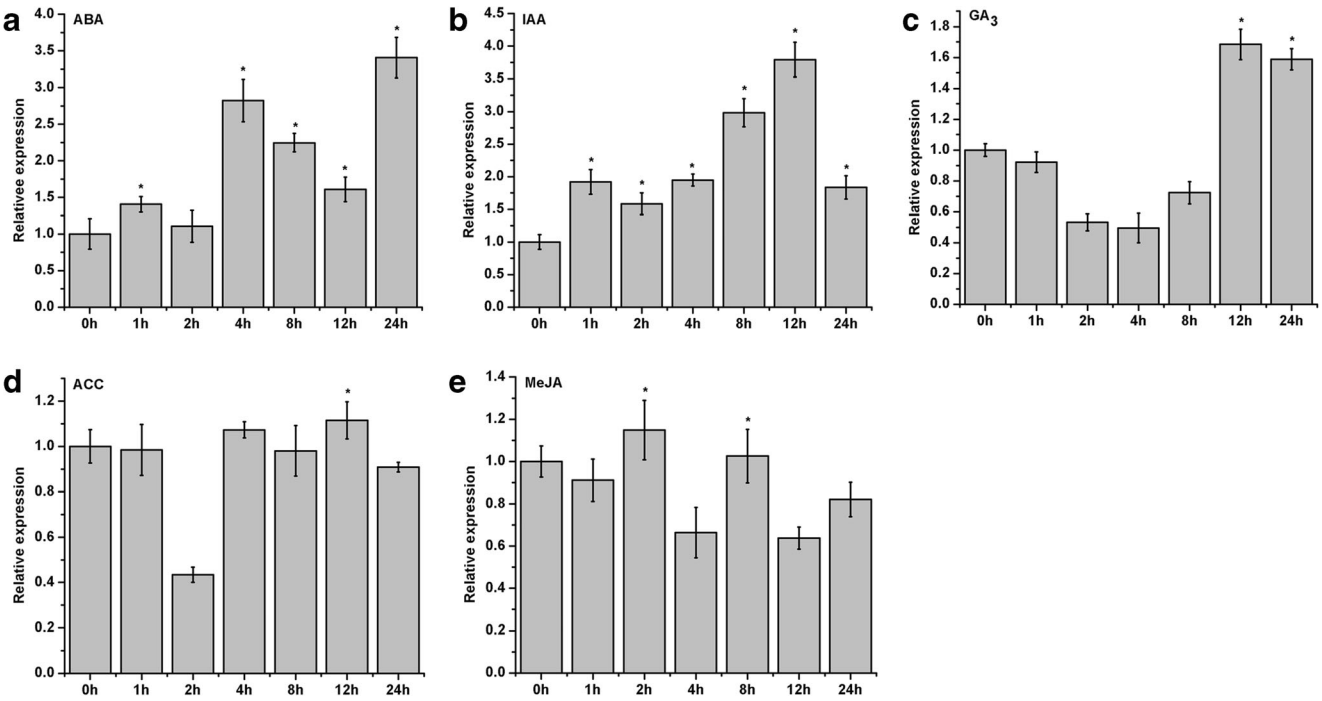

Fig. 5 Expression profile analysis of *SlGRAS15* in wild type (WT) tomato plants by qRT-PCR under various phytohormone treatments. The response of *SlGRAS15* expression to phytohormones **a** ABA, **b** IAA, **c** GA_3, **d** ACC, and **e** MeJA. The relative expression levels were normalized to 1 in untreated (mock) (sprayed with distilled water) plants (0 h). Each value represents the mean ± SE of three independent biological replicates ($n = 3$). Asterisks indicate a significant difference ($P < 0.05$) between untreated plants and treated plants

Discussion

Here, *SlGRAS15* gene a member of SCR subfamily has been identified and studied. Among the three members of this subfamily none of them have been studied in tomato. Expression analysis of *SlGRAS15* in different parts of tomato plant showed that it highly accumulates in roots, young leaves, and stem. This led us to hypothesize that *SlGRAS15* may function during the growth and development of tomato. Suppression of *SlGRAS15* in tomato altered plant morphological features, response to phytohormones treatment ABA, IAA, and GA_3, phase transition and significantly decreased the plant height and leaf size, extensive branching, increases leaf number, and petiolules length.

In our study, we observed that the transcript accumulation of *SlGRAS15* was affected by phytohormones treatment and had higher sensitivity to ABA, IAA, and GA_3 than the WT plants at seedlings stage (Fig. S3). The plant growth hormone ABA play key role in altering several aspects of plant developmental processes, like seed germination, seed dormancy, and growth of seedlings. ABA can also function to increase leaf abscission, senescence of leaf, and flower (Zhou et al. 2009). The qRT-PCR analysis showed that the transcripts of *SlGRAS15* mRNA gradually increased at the beginning, while increased slightly at 12 h after ABA treatment. These results shows that silencing *SlGRAS15* plays an important role in regulating plant responses to ABA during seedlings growth. Furthermore, the major constituents such as TFs, which are especially involved in the ABA signaling pathway can affect the sensitivity to ABA exposure (Waseem et al. 2019). The data revealed the involvement of *SlGRAS15* in the growth of roots, leaf, stem, and mediating hormone signaling.

The endogenous GA_3 contents were decreased in *SlGRAS15*-RNAi lines than WT (Fig. 7a). In *SlGRAS15*-RNAi lines, the 4 internodes length were smaller than WT plants, demonstrating that the dwarf transgenic lines depicted the shortening of internodal length (Fig. 3a–h), indicating that the dwarf phenotypes may be due to reduced endogenous bioactive GA_3 and short internodal length. To further confirm this phenotype, exogenous application of 100 μM GA_3 restored the plant height to WT levels (Fig. 7b, c). Therefore, we supposed that the low concentration of GAs might be responsible for these phenotypes. For the confirmation of this hypothesis, qRT-PCR analyses were performed to evaluate the transcript level of genes related to GAs production in the stem of *SlGRAS15*-RNAi lines and WT. The results revealed that the expression level of genes related to GA biosynthesis comprising *KAO*, *CPS*, *GA3ox1,* and *GA3ox2* were inhibited significantly in *SlGRAS15*-RNAi lines. Moreover, the *SEDILLA*-a negative regulator of GAs signaling was induced but *SlGAST1* (Ding et al. 2013) (tomato gibberellin stimulated transcript1) transcript accumulation reduced in *SlGRAS15*-RNAi lines. Conversely, we also evaluated the transcript accumulation of genes related to GA inactivation (*SlGA2ox1*, –*2*); prominent upregulation was observed in *SlGRAS15*-RNAi lines (Fig. 6). These results point out that *SlGRAS15*-downregulation may affect the tomato *DELLA* metabolism, afterwards effects the development of cell and consequently led to the GA unresponsive short plant phenotype of *SlGRAS15*-RNAi lines. It is well reported that the molecular phenomenon underlying the feedback regulation of GA metabolism is operated by *DELLA* proteins (Hou et al. 2008). *DELLAs* positively regulate the expression of *GA 3*-oxidases genes*;* however, *GA 2*-oxidase genes expression is suppressed. *DELLAs*

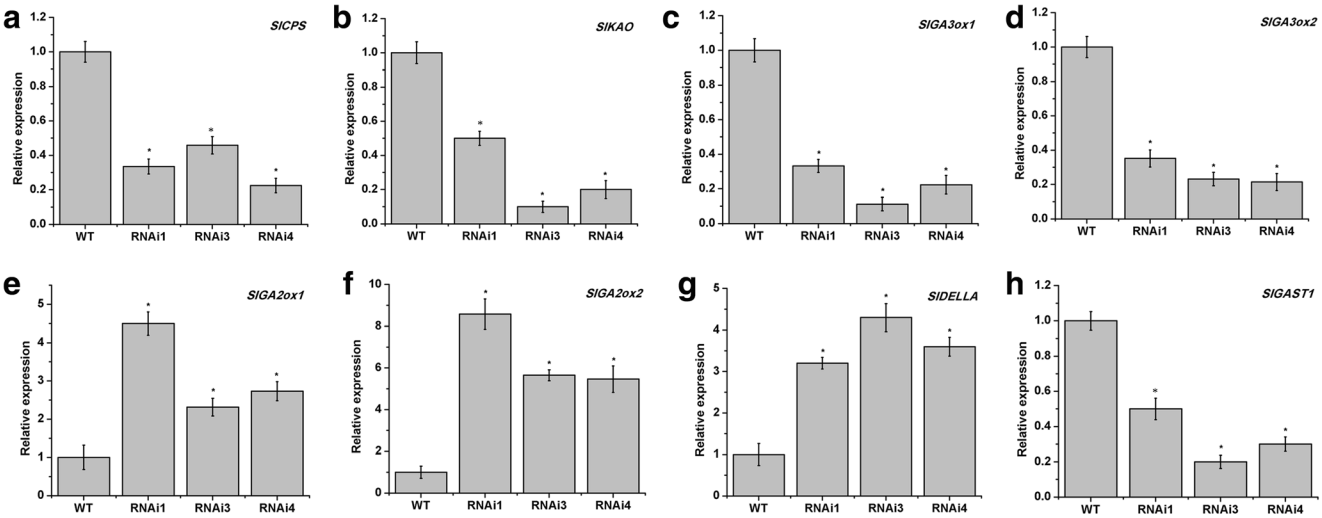

Fig. 6 Transcript levels of genes involved in GA biosynthesis, catabolism, and signal transduction pathway in wild type (WT) and *SlGRAS15*-silenced plants. **a**–**h** Expression levels of *SlCPS*, *SlKAO*, *SlGA3ox1*, *GA3ox2*, *SlGA2ox1, SlGA2ox2*, *SlDELLA*, and *SlGAST1* in WT and transgenic lines, respectively. Each value represents the mean ± SD of three independent biological replicates ($n = 3$). Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines

expression level is also exposed to negative feedback mechanism by their own protein products (Middleton et al. 2012). In *SlGRAS15*-silenced plants, we witnessed the same phenomena, including reduced transcripts of *SlGA3ox1* mRNA accumulation, increased levels of *DELLA* and *SlGA2ox1* transcripts. There by we speculate that downregulation of *SlGRAS15* might suppress the *DELLA* protein degradation in tomato plants, which is involved in changing the expression of genes related to gibberellin metabolism by the mechanism of positive and negative feedback. So, our results shows that silencing of *SlGRAS15* reduces expansion of leaf by lowering the transcript level of main genes involved in the biosynthesis of GA and triggering the genes involved in GA deactivation. On the other side the bioactive endogenous GA_3 in transgenic lines is lower as compared with WT plants (Fig. 7a). These results suggest that *SlGRAS15* plays an important role in tomato growth and development through interaction with the promoter region of GA biosynthesis genes in the GA signaling pathway, but whether these interactions are direct or indirect remains to be determined. In the present work, downregulation of *SlGRAS15* altered the responsiveness to IAA, led to auxin sensitivity, and altered the abundance of transcripts related to auxin biosynthesis and signaling (Figs. 5b and S3). This is compelling evidence that *SlGRAS15* acts as a regulator of auxin and influences auxin and gibberellin homeostasis.

Moreover, we observed that *SlGRAS15*-RNAi lines showed comparatively small sized leaves with pointed margins than that of WT but the leaf index (length to width ratio) remained unchanged (fig. 3f). The leaf growth is a complex stepwise process of development under the control of various environmental and developmental factors which are defined by proliferation, expansion, and maturation phases (Falcone et al. 2007). Two genes belong to NAC TFs family *SlGOB* (*Goblet*) (Berger et al. 2009) and *SlNAM2* (Hendelman et al. 2013), are well known for their function to control the leaf margins. So we quantified the expression level of these two genes in WT and *SlGRAS15*-RNAi lines. Significant increase of them was recorded in *SlGRAS15*-RNAi lines when compared with WT plants (Fig. S2). Leaves of tomato endure prolonged morphogenesis even when compared with species having compound leaves, causing elaborate and inconstant leaf forms. Leaf shape in tomato varies among different-cultivars, different plants of the same cultivar, and even unlike leaves on the same plant is interesting (Pattanaik et al. 2014). According to our analysis, *SlGRAS15*-RNAi plants displayed increased number of leaf and shoot branches, specifying the vigorous vegetative growth of these plants than that of WT (Figs. 3 and S2).

Tomato transgenic plants carrying *SlBL*-RNAi expression, the origination of lateral meristems is blocked during shoot

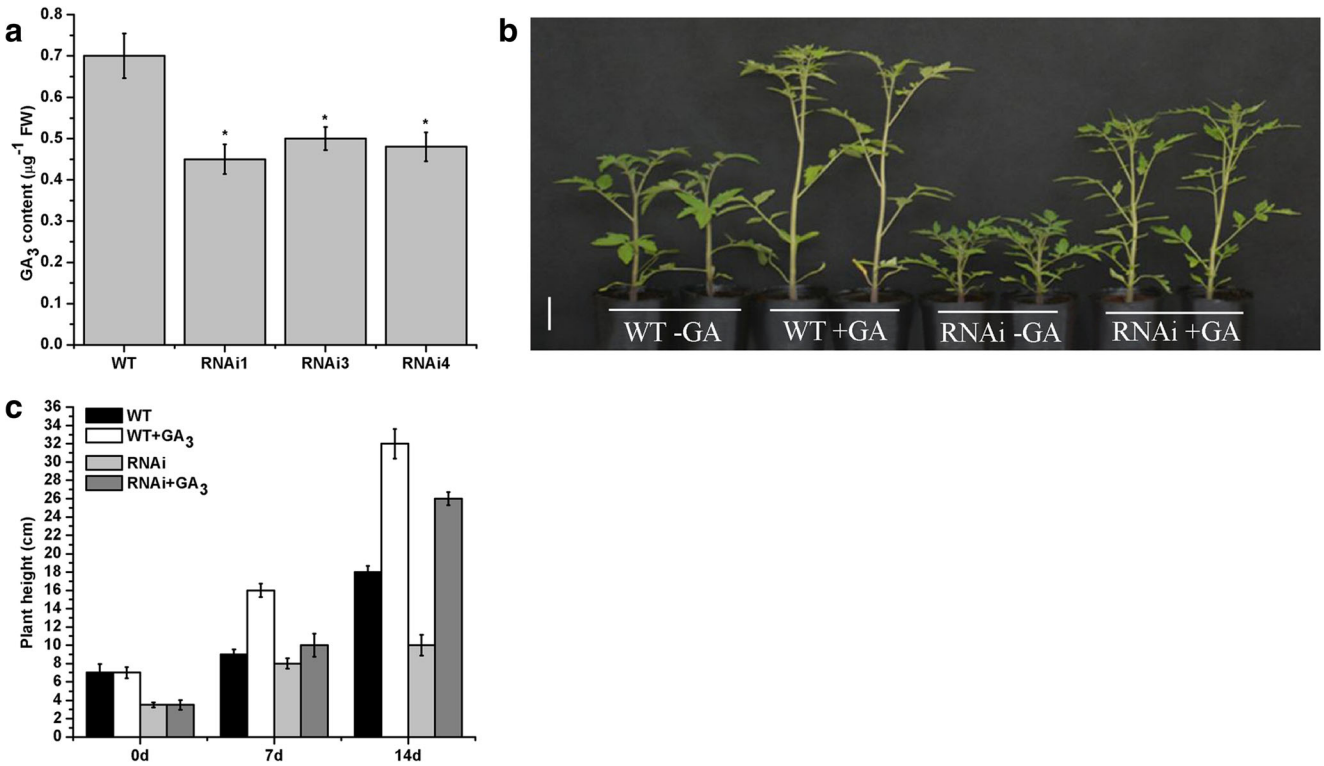

Fig. 7 Active GA_3 is lower in *SlGRAS15*-RNAi lines than in wild type (WT) plants; dwarf phenotype can also be rescued by spraying with 100 μM GA_3 and plant height of GA_3-treated plants. **a** The GA_3 content of WT and *SlGRAS15*-silenced lines. **b** The dwarf phenotype of *SlGRAS15* lines was rescued by spraying with 100 μM GA_3 on every 3rd day starting from 30 days after sowing up to 2 weeks; pictures were taken 14 days after treatment. **c** The plant height of GA_3-treated plants. Bars = 1 cm. Each value represents the mean ± SD of three independent biological replicates ($n = 3$). Asterisks indicate a significant difference ($P < 0.05$) between WT and transgenic lines

development and inflorescence, which leads to an intense decrease in the number of lateral axes (Silva et al. 2014). Therefore we assessed the transcript level of *SlBL* (Mauricio and Rausher 1997; Silva et al. 2014) in WT and *SlGRAS15*-RNAi lines, where *SlGRAS15*-RNAi lines showed an increased expression of *SlBL* in the axillary buds, showing that *SlGRAS15* gene may positively regulate the transcript level of *SlBL* (Fig. S2). In tomato plants the loss of function mutation of *SlBRC1b* leads to enlarged branch out growth, suggesting that *SlBRC1b* play a key role in suppression of shoot branch (Silva et al. 2014). The transcript level of *SlBRC1b* which act as shoot branch suppressor was also reduced in the axillary buds of *SlGRAS15*-RNAi lines. In angiosperms, shoot branching mainly defines the complete plant architecture and affects the key features of plant life, include plant height, nutrients distribution, and visibility for pollinators.

In conclusion, we have identified an important plant regulator, *SlGRAS15* in tomato. Results obtained from studies performed enable us to propose a linkage between *SlGRAS15* gene and manipulation of plant architecture, response to growth hormones, plant height, and leaf number by downregulating the main GA biosynthesis genes and stimulating the GA deactivating genes in tomato. Taken together, we highlight that *SlGRAS15* is an essential regulator involved in vegetative developmental processes. Our findings provide a valuable opportunity to deepen understanding on the genetic mechanism underlying significant tomato agronomic traits.

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Author's contributions M.N designed and managed the research; M.N., M.W., Z.Z., and L.Z performed research and revised the manuscript; M.N. wrote the paper. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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