Tomato SIBES1.8 influences leaf morphogenesis by mediating gibberellin metabolism and signaling

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Highlight

The BRI1-EMS-SUPPRESSOR 1 (BES1) family protein SIBES1.8 promotes leaf simplification by inhibiting gibberellin deactivation and physically interacting with DELLA protein in tomato.

Abstract

Leaf morphogenetic activity determines its shape diversity. However, our knowledge to the regulatory mechanism in maintaining leaf morphogenetic capacity is still limited. In tomato, gibberellin (GA) negatively regulates leaf complexity by shortening the morphogenetic window. We here reported a tomato BRI1-EMS-SUPPRESSOR 1 (BES1) transcription factor, SIBES1.8, that promoted the simplification of leaf pattern in a similar manner as GA functions. Enhanced level of SIBES1.8 dramatically decreased the sensibility of tomato to GA whereas increased the sensibility to the GA biosynthesis inhibitor, PAC. In line with the phenotypic observation, the endogenous bioactive GA contents were increased in OE-*SIBES1.8* lines, which certainly promoted the degradation of the GA signaling negative regulator, SIDELLA. Moreover, transcriptomic analysis uncovered a set of overlapping genomic targets of SIBES1.8 and GA, and most of them were regulated in the same way. Expression studies showed the repression of SIBES1.8 to the transcriptions of two GA deactivated genes, *SlGA2ox2* and *SlGA2ox6*, and one GA receptor, *SlGID1b-1*. Further experiments confirmed the direct regulation of SIBES1.8 to their promoters. On the other hand, SIDELLA physically interacted with SIBES1.8 and further inhibited its transcriptional regulation activity by abolishing SIBES1.8-DNA binding. Conclusively, by mediating GA deactivation and signaling, SIBES1.8 greatly influenced tomato leaf morphogenesis.

21

Keywords: BES1 transcription factor, leaf morphogenesis, gibberellin metabolism, gibberellin signaling, DELLA, tomato (*Solanum lycopersicum*).

24 Introduction

25 Leaf is a vital plant organ not only because of its functions as a solar panel that
 26 utilizing light energy to converts carbon dioxide into carbohydrates, but also for the
 27 integration of internal and external signals in response to various environmental
 28 information (Du *et al.*, 2018). Leaf primordia initiate from the peripheral zone (PZ) of
 29 shoot apical meristem (SAM), then the three axes of polar growth, that are the
 30 adaxial-abaxial, proximal-distal and medio-lateral axes, are gradually established
 31 (Byrne, 2012; Du *et al.*, 2018). The margins of leaf primordia maintain a transient
 32 window of meristematic activity, allowing the production of leaflets, lobes and
 33 serrations, thus fine-tuning of this window enables the variability in leaf patterning
 34 (Alvarez *et al.*, 2016; Israeli *et al.*, 2021). According to the number of blade, leaves
 35 are classified as simple leaf that only has a single blade and compound leaf that owns
 36 several separate blades attached to a common rachis (Efroni *et al.*, 2010). Tomato
 37 (*Solanum lycopersicum*) is a typical model plant that possesses compound leaves,
 38 which are composed of four kinds of leaflets: terminal leaflet (TL), primary lateral
 39 leaflet (PLL), secondary lateral leaflet (SLL) and intercalary leaflet (IL), and the lobes
 40 or serrations can be found in the margin of leaflet. There are usually one TL and three
 41 pairs of PLLs in tomato leaf branch. SLL develops from the petiolule of PLL, whereas
 42 IL develops later from the rachis between PLL, and their numbers are diverse and
 43 indeterminate (Goliber *et al.*, 1999).

44 The initiation, differentiation and maturation of plant leaf are controlled by the
 45 combination of transcriptional regulators, microRNAs and phytohormones. The
 46 initiation of leaf primordia is required for the presence of auxin maxima, which are
 47 established by the auxin efflux carrier PINFORMED1 (PIN1) (Reinhardt *et al.*, 2000;
 48 Reinhardt *et al.*, 2003). Late study reported that auxin induced the expression of a
 49 tomato ortholog of Arabidopsis *DORNROSCHE* (*DRN*) gene, *LEAFLESS* (*LFS*),
 50 which is necessary for leaf initiation, to specify primordium cells in the PZ (Capua
 51 and Eshed, 2017). Besides, many transcription factors are involved in the regulation
 52 of leaf initiation, including class I KNOTTED1-like homeobox proteins (KNOXI) and

the ASYMMETRIC LEAVES1 (AS1)/ROUGH SHEATH2 (RS2)/PHANTASTICA (ARP) MYB-domain transcription factors (Kerstetter *et al.*, 1997; Byrne *et al.*, 2000; Lodha *et al.*, 2013). During leaf patterning, a set of regulating factors including MONOPTEROS (MP, also named ARF5), GOBLET (GOB), LYRATE (LYR) and REDUCED COMPLEXITY (RCO) coordinately modulate the formation and shaping of leaflet in parallel different aspects (Vlad *et al.*, 2014; Israeli *et al.*, 2021). Leaf patterning is also greatly regulated by several miRNA-target modules. For instance, miR165/166 contributes to the establishment of leaf adaxial-abaxial polarity by targeting *HD-ZIP* □ mRNAs (Tatematsu *et al.*, 2015; Merelo *et al.*, 2016), miR319 and miR396 antagonistically regulate leaf marginal cell proliferation by targeting the class □ TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR (TCP) and GROWTH-REGULATING FACTORS (GRFs) respectively (Ori *et al.*, 2007; Rodriguez *et al.*, 2010; Tsukaya, 2018).

Leaf morphogenesis is also regulated by several phytohormones (Shwartz *et al.*, 2016). Among them, Gibberellin (GA) is shown to reduce the complexity of tomato leaf. Exogenous GA application promotes the maturation of tomato leaf and inhibits the formation of leaflets. Similarly, leaves of the *procera* (*pro*) mutant, which sustained mutation in the single tomato DELLA gene and showed constitutive GA response, bear fewer leaflets with smooth margins (Jasinski *et al.*, 2008; Lombardi-Crestana *et al.*, 2012; Shwartz *et al.*, 2016). This leaf simplification caused by increased GA levels or responses indicates the role of GA in shortening the morphogenetic window during leaf formation. Some leaf morphogenesis regulators are shown to function in mediating GA dynamics. In tomato, the CIN-TCP transcription factor LANCEOLATE (LA) accelerates leaf maturation and simplifies leaf shape partially by positively regulating GA levels (Yanai *et al.*, 2011). By contrast, KNOXI protein antagonizes GA activity by repressing the expression of GA biosynthesis gene *SLGA20ox1* in regulating leaf morphogenesis (Jasinski *et al.*, 2008).

In addition to GAs, Brassinosteroids (BRs) are another kind of plant growth-promoting hormone with functions in leaf development by promoting cell

82 elongation and differentiation (Mitchell *et al.*, 1970; Grove *et al.*, 1979). BR
83 biosynthetic and signaling mutants indeed show abnormal leaf patterning (Koka *et al.*,
84 2000; Zhiponova *et al.*, 2013). The crosstalk between GAs and BRs was well
85 explored in the last decade. In Arabidopsis, the negative regulators of GA signaling,
86 DELLA proteins, physically interacted with the dephosphorylated BRASSINAZOLE
87 RESISTANT1 (BZR1), the key positive regulator of BR signaling (Bai *et al.*, 2012;
88 Gallego-Bartolomé *et al.*, 2012; Li *et al.*, 2012). Moreover, the transcriptional
89 regulation activity of BZR1 was inhibited by the interaction with DELLA proteins
90 (Bai *et al.*, 2012; Gallego-Bartolomé *et al.*, 2012; Li *et al.*, 2012). On the other hand,
91 genetic and biochemical evidences suggested that BZR1 and
92 BRI1-EMS-SUPPRESSOR 1 (BES1) affected GA levels by directly regulating the
93 expression of GA biosynthetic genes *GA20ox* and *GA3ox* in Arabidopsis and rice
94 (Tong *et al.*, 2014; Unterholzner *et al.*, 2015). This integration of GA and BR both on
95 signaling level and hormone level allowed the elaborated regulation of plant growth
96 and leaf development.

97 Here, we reported a tomato BES1 family member, SIBES1.8, that can influence
98 leaf morphogenesis by mediating GA deactivation and signaling. Overexpression of
99 *SIBES1.8* in tomato significantly reduced the leaf complexity, which mimicked the
100 functions of GA in regulating leaf development. Indeed, the endogenous bioactive GA
101 contents were increased in OE-*SIBES1.8* leaves because of the transcriptional
102 repression of *SIGA2ox2* and *SIGA2ox6* by SIBES1.8, which further resulted in the
103 degradation of SIDELLA, hence the GA signaling was enhanced in OE-*SIBES1.8*
104 lines. On the other hand, the transcriptional regulation ability of SIBES1.8 was
105 inhibited by SIDELLA interaction, and such inhibition was released by the
106 SIBES1.8-promoted degradation of SIDELLA, which further enhanced GA signaling
107 in OE-*SIBES1.8* lines. Together, our results provide new insight into the crosstalk
108 between BES1 transcription factors and GA levels or responses in regulating leaf
109 morphogenesis.

110

111 **Materials and Methods**

112 ***Plant material growth conditions and generation of SIBES1.8 over-expressed lines***

113 The coding sequence of *SIBES1.8* was cloned into K303 binary vector under the
114 promotion of CaMV 35S promoter (Ren *et al.*, 2011; Liu *et al.*, 2020). The
115 recombined expression vector was transformed into *Agrobacterium tumefaciens* strain
116 GV3101 followed by cotyledon transformation according to V J Chetty *et al.* (2013)
117 in tomato (*Solanum lycopersicum*) cv. Micro-Tom and Ailsa Craig background to
118 generate the OE-*SIBES1.8* lines. Positive transgenic lines were screened by
119 kanamycin and PCR detection, and the relative mRNA level of *SIBES1.8* in positive
120 lines was detected by quantitative real-time polymerase chain reaction (qRT-PCR) in
121 T2 generation. All tomato plants were cultivated in greenhouse with 16/8 h light/dark
122 cycle, 25/20 °C day/night temperature and 60% relative humidity.

123

124 ***Observation of leaf pattern by scanning electron microscopy (SEM)***

125 The extra leaves of WT and OE-*SIBES1.8* plants at 15 DPG were hand-dissected to
126 expose the leaf primordium. The shoots of seedlings were subsequently immersed into
127 FAGE solution (50% absolute ethanol, 10% formaldehyde, 5% acetic acid and 0.72%
128 glutaraldehyde) immediately for one day followed by dehydrating in an ethanol series
129 from 30% to 100% over five days. The prepared samples were critically point dried
130 and coated with gold. Finally, the images were taken by a FEI Inspect S50 scanning
131 electron microscope.

132

133 ***Gibberellin A3 (GA₃) and paclobutrazol (PAC) treatment***

134 GA₃ and PAC (Sigma, USA) were dissolved in absolute methanol. For GA₃ and
135 PAC treatment, tomato seeds were germinated on MS/2 medium containing GA₃ (10
136 µM and 50 µM) or PAC (5 µM) respectively after sterilization. The control seeds were
137 germinated on MS/2 medium containing the same solution without GA₃ and PAC.
138 After 10-days normal growth, the seedlings were transplanted to the soil and sprayed
139 with 100 µM GA₃ or 50 µM PAC solution twice a week. Plant samples were observed

140 or collected at the corresponding times as described in the context.

141

142 ***Measurement of endogenous GAs contents***

143 For measuring endogenous GAs contents, leaves of WT and OE-*SIBES1.8* plants at
144 5-week-old were harvested and frozen into liquid nitrogen immediately. The
145 measurement was conducted by Wuhan Metware Biotechnology Co., Ltd. (Wuhan,
146 China) based on the AB Sciex QTRAP 6500 LC-MS/MS platform. Briefly, leaf
147 samples were ground using a mixer mill (MM 400, Retsch, Germany) to powder. GAs
148 were extracted with 70% (v/v) acetonitrile from 500 mg powder. After adding the
149 internal standards, 100 μ L trimethylamine and 100 μ L BPTAB, the resulting solution
150 was vortexed, incubated at 90 $^{\circ}$ C for 1 hour, and evaporated to dryness under nitrogen
151 gas stream followed by redissolving in 1 mL 70% (v/v) acetonitrile. The extracts were
152 filtered through 0.22 μ m filter and then analyzed using an UPLC-ESI-MS/MS system
153 (UPLC, ExionLCTM AD, <https://sciex.com.cn/>; MS, Applied Biosystems 6500 Triple
154 Quadrupole, <https://sciex.com.cn/>). Finally, the output data was analyzed by Analyst
155 1.6.3 software.

156

157 ***RNA extraction, cDNA synthesis and qRT-PCR analysis***

158 Total RNA was extracted by using the RNeasy Plant Mini Kit (Qiagen, Germany)
159 and treated with RNase-free DNase (Qiagen, Germany). The integrity and
160 concentration of total RNA were detected by agarose gel electrophoresis and
161 NanoDrop 1000 (Thermo, USA) respectively. After that, the PrimeScriptTM RT
162 reagent Kit with gDNA Eraser (TAKARA, Japan) was used to synthesize the
163 first-strand cDNA with 1 μ g total RNA as the template. The cDNA products were
164 diluted to 5-fold with deionized water before use. qRT-PCR was performed by TB
165 Green[®] Premix Ex TaqTM (Tli RNaseH Plus) (TAKARA, Japan) on the CFX96
166 TouchTM Real-Time PCR Detection System (BIO-RAD, USA) according to the
167 manufacturer's instructions. The relative fold change was calculated by the $2^{-\Delta\Delta C_t}$
168 method. *SIUBI* (Solyc01g056940) was used as the internal reference gene and all

169 primers used in qRT-PCR detection were listed in Supplementary Table S1.

170

171 ***RNA sequencing and data processing***

172 The fourth leaf primordium (P4) of WT and OE-*SIBES1.8* seedlings at 16 DPG
173 (that is L6 presented in Fig. 2) treated with or without 50 μ M GA₃ was microdissected
174 with a surgical blade and frozen into liquid nitrogen immediately, which was regarded
175 as the leaf samples used in RNA sequencing. At least 30 leaf primordia were collected
176 for each sample, and three independent samples were sequenced for each group. The
177 total RNA was extracted by using the PicoPureTM RNA Extraction kit (ARCTURUS,
178 USA) according to the user guide. The concentration of total RNA was measured by
179 NanoDrop 1000 (Thermo, USA). The integrity of total RNA was detected by agarose
180 gel electrophoresis and 2100 Bioanalyser (Agilent, USA) and only those RNA
181 samples showed clear bands and high RNA Integrity Number were conducted
182 sequencing library. The RNA-seq was carried out in Majorbio (Shanghai Majorbio
183 Bio-pharm Technology, Ltd, China) with the Illumina HiSeq xten/Novaseq 6000
184 sequencer. The raw data were trimmed and qualified by SeqPrep and Sickle with
185 default parameters. The generated clean reads were subsequently mapped to the
186 tomato genome in the Solanaceae Genomics Network (<http://solgenomics.net/>) by
187 using HISAT2 software. Finally, DESeq2 was used to analyze the differentially
188 expressed genes (DEGs) with fold change (FC) > 2 and adjust *P*-value (*Q*-value) <
189 0.05.

190

191 ***Electrophoretic mobility shift assay (EMSA)***

192 Full length of *SIBES1.8* and *SIDELLA* coding sequences were cloned into
193 pGEX-4T-1 plasmid. The recombinant plasmids were expressed in *Escherichia coli*
194 strain Rosetta2 BL21 cells (Transgen, China) to produce the GST fused proteins. The
195 fused proteins were purified with the GST-tagged protein purification kit (Clontech,
196 USA) according to the manufacturer's instructions. The designed probes of *SIGA2ox2*,
197 *SIGA2ox6* and *SIGID1b-1* were biotin labeled following the instruction of Light

Shift[®] Chemiluminescent EMSA Kit (Thermo, USA). The mutated biotin probes that had an AAAAAA fragment instead of G-box were used to confirm the specific binding and the unlabeled probes were used as the competitor. For assessing the effect of SIDELLA to the DNA binding ability of SIBES1.8, SIBES1.8 and SIDELLA proteins were mixed before EMSA reaction. All native and mutated probes were listed in Supplementary Table S1.

Yeast one-hybrid (Y1H) assays

The promoter fragments of *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1* were constructed into pAbAi plasmid and transformed into Y1HGold yeast strain under the guidance of Yeastmaker[™] Yeast Transformation System 2 (TAKARA, Japan). The recombinant yeast strain was confirmed by PCR conducted with Matchmaker[™] Insert Check PCR Mix (TAKARA, Japan). The full length of *SIBES1.8* coding sequence was cloned into pGADT7 plasmid and subsequently transformed into the recombinant yeast strain. The empty pGADT7 plasmid was used as negative control. The transformants were cultivated on SD/-Leu or SD/-Leu/AbA (Aureobasidin A, Clontech, USA) medium for three days. DNA-protein interactions between SIBES1.8 and promoter fragments were determined by the growth of colony.

Dual-luciferase assays

To explore the direct transcriptional regulation of SIBES1.8 to *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1*, full length of *SIBES1.8* coding sequence was cloned into pGreen □ 62-SK to generate the effector, and promoter fragments of *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1* were constructed into pGreen II 0800-LUC respectively to generate the reporters (Hellens *et al.*, 2005). After transiently co-expressed the effector and reporter in tobacco leaf for one-day darkness and another two-days normal growth, the expression of *LUC* and *REN* were detected by the Dual-Luciferase Reporter Assay System (Promega, USA). At least six biological replicates were measured, and the detection was performed independently for two times with similar

227 results.

228 To explore the effect of *SIDELLA* on the transcriptional repression activity of
229 *SIBES1.8*, full length of *SIBES1.8* and *SIDELLA* coding sequences were cloned into
230 pEAQ-GAL4BD vector respectively to generate the effectors. In reporter, the
231 expression of firefly luciferase (LUC) was under the control of GAL4-binding
232 element (5×GAL4), which was fused with the minimal TATA region of CaMV35S,
233 and the renilla luciferase (REN) promoted by CaMV35S was considered as the
234 internal control. The *SIBES1.8* and *SIDELLA* effectors were separately or together
235 co-expressed with the reporter in tobacco leaves. Finally, the *LUC* and *REN*
236 expression were detected by the Dual-Luciferase Reporter Assay System (Promega,
237 USA). At least six biological replicates were measured, and the detection was
238 performed independently for two times with similar results.

239

240 ***Immunoblot assay***

241 For immunoblot analyses, 16-days-old tomato seedlings were sprayed with 100 μM
242 GA₃ or 50 μM PAC solution for 3 hours, and then frozen into liquid nitrogen
243 immediately. Protein extracts were performed with the Plant Total Protein Extraction
244 Kit (Sigma, USA). Protein concentrations were measured using the BCA method.
245 Equal amounts of protein from each sample were boiled in 4× Laemmli protein
246 sample buffer (Bio-rad, USA) for 10 min followed by separating with 10%
247 SDS-PAGE. After a wet transfer to PVDF membranes, the anti-GAI rabbit antibody
248 and goat anti-rabbit IgG antibody (Agrisera, Sweden) were used to perform
249 immunoblot.

250

251 ***Yeast two-hybrid (Y2H) assays***

252 Full length of *SIBES1.8* and *SIDELLA* coding sequences were constructed into
253 pGBKT7 and pGADT7 as bait and prey respectively. The recombined plasmids were
254 co-transformed into Y2H Gold yeast cells. Culture mediums SD/-Leu/-Trp and
255 SD/-Ade/-His/-Leu/-Trp (Clontech, USA) with or without X-α-gal were used to select

the positive transformants.

Bimolecular fluorescence complementation (BiFC) assays

Full length of *SIBES1.8* and *SIDELLA* coding sequences were cloned into pXY104-YFP^C and pXY106-YFP^N (Yu *et al.*, 2008) respectively. The BiFC assays were performed as described previously (Luo *et al.*, 2014). In short, the recombined plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101 and co-injected into and expressed in tobacco epidermic cells. The recombined plasmids co-injected with corresponding empty YFP^N or YFP^C were used as negative control. After incubated in darkness for one day and in normal light condition for another two days, the fluorescence images were captured by the laser scanning confocal microscope (Leica TCS SP8, Germany).

Firefly luciferase complementation imaging (LCI) assays

Full length or truncated versions of *SIBES1.8* and *SIDELLA* were fused with nLUC or cLUC respectively. The LCI assays were conducted following the description by Chen *et al.* (2008). Briefly, the recombined plasmids were transformed into *Agrobacterium tumefaciens* strain GV3101. The fused *SIBES1.8*-nLUC transformants were transiently co-expressed with cLUC-*SIDELLA* transformants in tobacco leaves. After incubated in darkness for one day and in normal light condition for another two days, the tobacco leaves were sprayed with one millimolar luciferin (Promega, USA) on the blade back and kept in dark at least for six minutes. Finally, the LCI images were captured by a low-light cooled CCD imaging apparatus (Alliance, UK). At least four leaves were observed for each combination.

Accession numbers

Arabidopsis genes are obtained from The Arabidopsis Information Resource (TAIR, <https://www.arabidopsis.org/>) under the following identities: *GAI*, AT1G14920; *RGA*, AT2G01570; *RGL1*, AT1G66350; *RGL2*, AT3G03450; *RGL3*, AT5G17490. Tomato

genes are collected from Solanaceae Genomics Database (<http://solgenomics.net/>) under the following accession numbers: *SIBES1.8*, Solyc10g076390; *SIGA2ox2*, Solyc07g056670; *SIGA2ox6*, Solyc01g058030; *SlGID1b-1*, Solyc09g074270; *SIDELLA*, Solyc11g011260; *SIGA2ox1*, Solyc05g053340; *SIGA2ox3*, Solyc01g079200; *SIGA2ox4*, Solyc07g061720; *SIGA2ox5*, Solyc07g061730; *SIGA2ox7*, Solyc02g070430; *SIGA2ox8*, Solyc08g016660; *SIGA2ox9*, Solyc10g007570; *SlGID1ac*, Solyc01g098390; *SlGID1b-2*, Solyc06g008870; *SISLY1*, Solyc04g078390; *SISNE*, Solyc07g047680; *SIUBI*, Solyc01g056940. The raw RNA-sequencing data are publicly available in the Genome Sequence Archive in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences with the accession number of CRA005324.

Results

SIBES1.8 mimics the effect of exogenous GA application in simplifying leaf complexity

Previously, we reported that *SIBES1.8* exhibited distinct gene structure compared to other *BES1* members in tomato and acted as a transcriptional repressor with floral tissues specific expression pattern (Su *et al.*, 2021). To explore the functions of *SIBES1.8* in plant development, we generated over-expressed (OE) lines driving by CaMV35S promoter in micro-tom background. Three OE-*SIBES1.8* lines (L4, L5 and L8) with dramatically increased *SIBES1.8* expressions were selected to analyze the phenotypic changes (Supplementary Fig. S1A). Remarkably, OE-*SIBES1.8* plants exhibited simpler leaf shape with smooth margin compared with wild type (WT) plants, and this phenotypic change occurred in early leaf primordium development (Fig. 1A-C). In WT leaf primordium, serrations were emerged from the margin of leaflet, while there was no such structure in OE-*SIBES1.8* leaf primordium (Fig. 1A, B). Consistent with this, the serration numbers of mature terminal leaflets and primary lateral leaflets were significantly decreased in OE-*SIBES1.8* leaves (Fig. 1C). Given that micro-tom is a dwarf cultivar of tomato and its leaves are simplified by the

314 mutation in the *SELF-PRUNING* (*SP*) and *DWARF* (*D*) genes, which are related with
 315 shoot apical meristem development and BR biosynthesis respectively (Martí *et al.*,
 316 2006), we also generated OE-*SIBES1.8* lines in *Ailsa Craig* background
 317 (Supplementary Fig. S1B), in which the leaf pattern remained complicated. Consistent
 318 with the observation in micro-tom background, OE-*SIBES1.8* leaves also showed
 319 simpler shape and smooth margin (Fig. 1D, Supplementary Fig. S2). The leaflets can
 320 be briefly classified into three groups: terminal leaflet and primary lateral leaflets (1st),
 321 secondary lateral leaflets (2nd) and intercalary leaflets (3rd) (Fig. 1E). The numbers
 322 of all of these three kinds of leaflets were decreased in OE-*SIBES1.8* leaves (Fig. 1F).
 323 On the contrary, we also generated *slbes1.8* mutants by CRISPR/Cas9 technology.
 324 Two homozygous lines with distinct mutations were selected to analyze the leaf
 325 pattern (Supplementary Fig. S3A), while no obvious phenotypic changes were
 326 observed (Supplementary Fig. S3B), and the leaflet serration numbers of the 5th leaf
 327 were not significantly influenced in *slbes1.8* mutants (Supplementary Fig. S3C),
 328 indicating that knockout of *SIBES1.8* didn't affect the normal growth of tomato leaf
 329 development.

330 The decreased leaf complexity in OE-*SIBES1.8* plant is reminiscent of the functions
 331 of GA in leaf development that increased GA levels or responses could predominantly
 332 simplify leaf shape (Jasinski *et al.*, 2008; Yanai *et al.*, 2011; Lombardi-Crestana *et al.*,
 333 2012; Livne *et al.*, 2015; Tomlinson *et al.*, 2019). To compare the similarity of leaf
 334 phenotypic changes caused by OE-*SIBES1.8* and GA application, we treated wild type
 335 seedlings with GA₃ and observed the leaf shape. The results showed that WT leaves
 336 possessed several serrations in the margin (Fig. 2A). This serration structure can be
 337 firstly observed in L6 (the 4th leaf primordium, P4), while no such structure can be
 338 found after applying exogenous GA₃ (Fig. 2A, B). In OE-*SIBES1.8* seedlings, there is
 339 also no serration structure taken shape (Fig. 2C). Phenotypic observation in mature
 340 leaves further confirmed the consistent roles of GA₃ and *SIBES1.8* in leaf
 341 development (Supplementary Fig. S4). These results indicate that OE-*SIBES1.8*
 342 altered the leaf morphogenesis in a manner that similar to GA application.

343

344 ***SIBES1.8 decreases the sensibility of tomato to exogenous GA₃ while increases the***
 345 ***sensibility to PAC***

346 The similarity in shaping leaf promotes the possibility that SIBES1.8 may have
 347 direct or indirect correlation with GA. We first analyzed the potential *cis*-elements in
 348 the promoter of *SIBES1.8* and found that there was a GA responsive TATC-box (Fig.
 349 3A). Moreover, qRT-PCR detection verified the induction of GA₃ to the transcription
 350 of *SIBES1.8* (Fig. 3B). The primary root of OE-*SIBES1.8* seedlings was shorter than
 351 WT and was not influenced by GA₃ treatment (Fig. 3C, D). In addition, the most
 352 obvious phenotypic change of tomato seedling after GA₃ treatment is the elongation
 353 of first shoot (Fig. 3C). Surprisingly, the GA-induced first shoot elongation was
 354 suppressed in OE-*SIBES1.8* lines (Fig. 3C, E). These results suggested that SIBES1.8
 355 decreased the sensibility of tomato seedling to exogenous GA₃. On the other hand,
 356 paclobutrazol (PAC, an inhibitor of GA biosynthesis) treatment could suppress plant
 357 growth and produce smaller leaves (Fig. 3F). While the growth suppression of PAC to
 358 OE-*SIBES1.8* plants was much stronger than WT plants (Fig. 3F). These results
 359 indicated that SIBES1.8 increased the sensibility of tomato to PAC. Noticeably, PAC
 360 treatment partly recovered the serration in OE-*SIBES1.8* leaf margin (Fig. 3F), which
 361 further tied SIBES1.8 functions with GA level or response in leaf development.

362

363 ***SIBES1.8 increases endogenous bioactive GA contents in tomato leaf***

364 To assess the effect of OE-*SIBES1.8* to GA levels, we detected the endogenous GA
 365 contents of WT and OE-*SIBES1.8* leaves. According to the GA biosynthesis pathway,
 366 we measured the endogenous contents of 16 kinds of GAs in mature leaves, including
 367 GA₁₅, GA₉, GA₅₁, GA₄, GA₃₄, GA₂₀, GA₁, GA₈, GA₅, GA₃, GA₇, GA₂₄, GA₅₃, GA₁₉,
 368 GA₂₉ and GA₆ (Fig. 4A, B, Supplementary Table S2). Among them, the contents of
 369 GA₂₄, GA₅₃, GA₁₉, GA₂₉ and GA₆ were too low to detect. In general, total GAs
 370 contents were not significantly changed in OE-*SIBES1.8* leaves (Fig. 4C), while the
 371 dominant two kinds of GAs, bioactive GA₃ and inactive GA₈, were increased and

decreased respectively (Fig. 4B, D). Furthermore, the other two bioactive GAs, GA₁ and GA₄ were unchanged and increased respectively in OE-*SIBES1.8* leaves (Fig. 4E). These results suggested that the increased bioactive GA levels may account for the decreased complexity of OE-*SIBES1.8* leaf.

SIBES1.8 and GA regulate overlapping genomic targets

To view insight of the genome-wide transcriptomic profiling of OE-*SIBES1.8* plants, we performed RNA-sequencing for WT and OE-*SIBES1.8* leaf primordium at P4 stage. With the criterions of fold change (FC) > 2 and adjust *P*-value (*Q*) < 0.05, a total of 259 and 297 differentially expressed genes (DEGs) were influenced by GA₃ application and OE-*SIBES1.8* respectively (Fig. 5A, Supplementary Table S3, 4). Among them, 42 DEGs were regulated by both GA₃ and *SIBES1.8*, which promoted the close relationship between GA₃ and *SIBES1.8* (Fig. 5B, Supplementary Table S5). If *SIBES1.8* mimics the functions of GA in leaf development, *SIBES1.8* should affect the expression of genes that influenced by GA in a similar manner. Indeed, by analyzing the expression profiles of these 42 co-DEGs, we found that 36 DEGs (85.7%) were affected in the same way by GA₃ and OE-*SIBES1.8* (Fig. 5C, group □), whereas only six DEGs were influenced in the opposite way (Fig. 5C, group □). Notably, when compared the expression of these co-DEGs in GA₃ treated OE-*SIBES1.8* leaves with mocked WT leaves (OE GA₃ vs WT Mock), the expression changes displayed a superimposed effect of OE-*SIBES1.8* and GA₃ treatment, which suggested that on the basis of exogenous GA₃ application, OE-*SIBES1.8* further strengthened GA signaling (Fig. 5C). Meanwhile, by comparing the expression of these 42 DEGs in WT and OE-*SIBES1.8* leaves after GA₃ treatment, we found that 28 DEGs (66.7%, group □) exhibited weaker expression changes in OE-*SIBES1.8* leaves than that in WT (Fig. 5D), suggesting that OE-*SIBES1.8* repressed their responses to GA₃ application, which further supported our previous conclusion that *SIBES1.8* decreased the sensibility of tomato to exogenous GA₃. Collectively, OE-*SIBES1.8* and GA₃ application influenced a mass of common targets in the similar manner.

401

402 ***SIBES1.8 binds to and represses the activities of SIGA2ox2, SIGA2ox6 and***
 403 ***SIGID1b-1 promoters***

404 To elucidate the regulatory mechanism of SIBES1.8 in regulating leaf development
 405 by mediating GA levels or responses, we carefully analyzed all DEGs affected by
 406 OE-*SIBES1.8* and GA₃ application, and found that merely none of them was reported
 407 to involve in GA biosynthesis or signaling (Supplementary Table S3, 4). This may be
 408 caused by the very early developmental status of RNA-seq samples (the 4th leaf
 409 primordium of seedlings at 16DPG) that few of genes exhibited differential
 410 expression in this stage. Hence, we selected to explore the potential targets of
 411 SIBES1.8 by using the older leaves (that is L3 presented in Fig. 2) as the materials.

412 The increased bioactive GA contents could be caused by the up-regulation of GA
 413 biosynthetic genes or the down-regulation of GA deactivated genes. Given that
 414 SIBES1.8 acts as a transcriptional repressor (Su *et al.*, 2021), we mainly considered
 415 the repression of SIBES1.8 to the expression and responsiveness of *GA 2-oxidase*
 416 (*GA2ox*) genes, which are the best-characterized GA deactivated genes. The relative
 417 expressions of nine tomato *GA2ox* genes in WT and OE-*SIBES1.8* leaves with or
 418 without GA₃ treatment were explored by qRT-PCR. Overall, *SIGA2oxs* transcriptions
 419 were greatly induced by GA₃ in WT leaves as expected (Fig. 6A, B, Supplementary
 420 Fig. S5A). Among them, the expression levels of *SIGA2ox2* and *SIGA2ox6* were
 421 significantly reduced in OE-*SIBES1.8* leaves, and their responses to GA₃ application
 422 were also suppressed by OE-*SIBES1.8* (Fig. 6A, B), suggesting that SIBES1.8 may
 423 increase the bioactive GA contents through repressing the expressions of *SIGA2ox2*
 424 and *SIGA2ox6*. Meanwhile, we also detected the relative expression of genes involved
 425 in GA signaling (Fig. 6C, Supplementary Fig. S5B). Among them, *SIGID1b-1* showed
 426 reduced expression in OE-*SIBES1.8* leaves (Fig. 6C), indicating a potential direct
 427 regulation by SIBES1.8.

428 To confirm the direct regulation of SIBES1.8 to *SIGA2ox2*, *SIGA2ox6* and
 429 *SIGID1b-1*, we conducted electrophoretic mobility shift assay (EMSA), yeast

one-hybrid assay (Y1H) and dual-luciferase assay according to the existence of G-box (CACGTG), a well-identified binding motif of BES1 family (He *et al.*, 2005; Yin *et al.*, 2005), in the promoters of *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1* (Fig. 6D). EMSA results showed the specific binding of SIBES1.8 to the selected promoter fragments (Fig. 6E). Y1H results further confirmed the binding of SIBES1.8 to *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1* promoters (Fig. 6F). Moreover, we used dual-luciferase system to detect the effects of SIBES1.8 to the promoter activities in tobacco leaves and found that SIBES1.8 significantly repressed the activities of *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1* promoters (Fig. 6G, H). The transcriptional repression of SIBES1.8 to *SIGA2ox2* and *SIGA2ox6* may be the reason of increased bioactive GA contents in OE-*SIBES1.8* leaves, which should promote the degradation of SIDELLA protein. Indeed, by performing immunoblot, we confirmed the decreased level of SIDELLA protein in OE-*SIBES1.8* lines (Fig. 6I). Collectively, these results indicated that SIBES1.8 increased the endogenous bioactive GA levels by repressing the expression of *SIGA2ox2* and *SIGA2ox6*, which further promoted the degradation of SIDELLA protein, resulting in a similar effect with increased GA levels or responses in tomato leaf development.

447

SIDELLA physically interacts with SIBES1.8 and inhibits its transcriptional regulation ability by abolishing SIBES1.8-DNA binding

DELLA protein lacks a DNA-binding domain, which promotes the necessary of intermediate proteins for its functions (Yoshida *et al.*, 2014; Marín-de la Rosa *et al.*, 2015). In Arabidopsis, DELLAs were proved to physically interact with BES1/BZR1, which further inhibited the DNA binding ability of BZR1 (Bai *et al.*, 2012; Gallego-Bartolomé *et al.*, 2012; Li *et al.*, 2012). The characteristic domains of DELLA proteins were conserved in tomato and Arabidopsis (Supplementary Fig. S6), which raised the possibility that tomato DELLA protein may interact with SIBES1 transcription factors in a similar manner, including SIBES1.8. To prove this assumption, we investigated the protein-protein interaction between SIDELLA and

459 SIBES1.8 by yeast two-hybrid assay (Y2H), bimolecular fluorescence
460 complementation (BiFC) assay and firefly luciferase complementation imaging (LCI)
461 assay. The results showed that SIDE^{LLA} can truly interact with SIBES1.8 (Fig. 7A-C).
462 Furthermore, we explored the interacted domains of SIDE^{LLA} and SIBES1.8 by
463 generating a series of deletion constructs used in LCI assays according to the amino
464 acid sequence analysis (Supplementary Fig. S7A). The results showed that the
465 deletion of PEST domain of SIBES1.8 was sufficient to prevent the interaction with
466 SIDE^{LLA} (Supplementary Fig. S7B), and deletion of DELLA motif of SIDE^{LLA}
467 abolished the interaction with SIBES1.8 (Supplementary Fig. S7C), indicating that the
468 PEST domain of SIBES1.8 and the DELLA motif of SIDE^{LLA} were critical for their
469 protein-protein interaction.

470 We next analyzed the effect of SIDE^{LLA} interaction to the transcriptional
471 regulation activity of SIBES1.8 by GAL4-responsive reporter system in tobacco
472 leaves. Full length of *SIDE^{LLA}* and *SIBES1.8* coding sequences were fused with
473 GAL4BD respectively to generate the effectors, which were subsequently
474 co-expressed with reporter in tobacco leaves (Fig. 7D). As the transcriptional
475 activation control, VP16 greatly induced the expression of *LUC*, indicating the
476 effectiveness of this system. In line with our previous detection (Su *et al.*, 2021),
477 SIBES1.8 significantly repressed *LUC* expression, suggesting the transcriptional
478 repression activity. On the contrary, SIDE^{LLA} increased *LUC* expression and
479 exhibited transactivation activity, which was consistent with the rice DELLA protein,
480 SLR1 (Hirano *et al.*, 2012). Dramatically, SIDE^{LLA} antagonized the transcriptional
481 regulation activity of SIBES1.8 when they co-expressed together (Fig. 7E). Besides,
482 using EMSA, we confirmed that the bindings of SIBES1.8 to the promoter fragments
483 of *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1* were suppressed by the presence of
484 SIDE^{LLA} (Fig. 7F). Noticeably, SIDE^{LLA} was unable to bind these fragments in the
485 absence of SIBES1.8, as expected. Taken together, these results indicated that through
486 protein-protein interaction, SIDE^{LLA} can inhibit the transcriptional regulation
487 activity of SIBES1.8 by repressing its DNA binding ability.

488

489 **Discussion**

490 Leaf morphological diversity is achieved by fine-tuning the morphogenetic window
 491 during development that prolonged window resulted in complex leaf patterning
 492 whereas shortened window led to simpler leaf shape (Kierzkowski *et al.*, 2019).
 493 During this elaborated developmental process, several regulators acted coordinately to
 494 affect different aspects of leaf shape, ensuring the establishment of a suitable pattern
 495 (Du *et al.*, 2018; Israeli *et al.*, 2021). Hormones play critical roles in various aspects
 496 of plant development including leaf formation (Shwartz *et al.*, 2016). Among them,
 497 GA is known to reduce the complexity of leaf pattern in tomato. Tomato plants treated
 498 with exogenous GA or mutated in *DELLA* locus exhibited simplified leaf that with
 499 less leaflet production and smooth margin (Jasinski *et al.*, 2008; Lombardi-Crestana *et*
 500 *al.*, 2012; Livne *et al.*, 2015; Shwartz *et al.*, 2016; Tomlinson *et al.*, 2019). By
 501 investigating the early leaf development of tomato *DELLA* mutant *pro*, the
 502 simplification of *pro* leaf was caused by the combination of accelerated early
 503 developmental growth and delayed leaflet initiation (Jasinski *et al.*, 2008), indicating
 504 the effects of increased GA signaling in shortening leaf morphogenetic phase. Since
 505 the clarification of GA functions in leaf development, several regulators that can
 506 greatly influence leaf morphogenesis were reported to fulfill their roles by affecting
 507 GA homeostasis, such as LA and KNOXI (Jasinski *et al.*, 2008; Yanai *et al.*, 2011). In
 508 this research, we also identified a tomato leaf development regulator, *SIBES1.8*, that
 509 promoted leaf simplification by mediating GA homeostasis and signaling. We offered
 510 multiple lines of evidence to support this finding. First, OE-*SIBES1.8* lines in
 511 micro-tom and *Ailsa Craig* background presented less leaflet with smooth margin,
 512 which was highly resemble to GA-treated wild type leaves. Second, endogenous
 513 bioactive GA levels were increased in OE-*SIBES1.8* leaves, which subsequently led to
 514 the degradation of *SIDELLA* as expected. Third, OE-*SIBES1.8* and GA affected a set
 515 of overlapping targets in a similar manner. Fourth, *SIBES1.8* directly regulated the
 516 expression of *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1*. And fifth, *SIBES1.8* physically

517 interacted with SIDEELLA and were inhibited by such interaction in DNA binding
518 ability. In summary, we proposed a working model to depict the molecular mechanism
519 of SIBES1.8 in regulating tomato leaf morphogenesis by mediating GA deactivation
520 and signaling (Fig. 8). Although we determined the correlation of SIBES1.8 with GA,
521 the downstream targets of GA signaling that directly contribute to leaf development
522 remain largely unknown and need to be investigated. Also, new modifiers of leaf
523 pattern that affect variable aspects of leaf shape such as lobe/serration, leaflet number
524 and size, are worth to be discovered.

525 To identify the functions of SIBES1.8, we also generated CRISPR/Cas9 mutants for
526 it and expected the corresponding phenotypic changes to OE-*SIBES1.8* plants in leaf
527 development. We selected two homozygous lines with distinct mutations to observe
528 the leaf phenotype and failed to obtain obvious developmental differences
529 (Supplementary Fig. S3). Two reasons may explain this. First, the constitutive
530 expression level of *SIBES1.8* is very low, with an average of 0.86 TPM transcription
531 in wild type leaves, as a contrast, its transcription was increased to 1555.67 TPM in
532 OE-*SIBES1.8* leaves (Supplementary Table S4). The low content of SIBES1.8
533 contributed to a weak repression to *SIGA2ox2* and *SIGA2ox6* transcription that its
534 knockout was not enough to affect endogenous GA levels. Second, decreasing GA
535 levels or responses contributed a relative minor effect on leaf complexity. Tomato
536 mutants with reduced GA levels or responses generally sustained pleiotropic effects,
537 which made it difficult to interpret its effect on leaf formation (Koornneef *et al.*, 1990;
538 Jasinski *et al.*, 2008; Yanai *et al.*, 2011; Livne *et al.*, 2015; Tomlinson *et al.*, 2019).
539 Besides, specific reducing endogenous GA levels in leaf by overexpressing GA
540 deactivated gene *GA2ox* under the driving of leaf-specific promoter *pFIL* resulted in
541 more complex leaf pattern, while was moderate compared to the substantial
542 simplification effect caused by increasing GA levels or responses (Shwartz *et al.*,
543 2016).

544 BR and GA are known as two principal groups of plant growth-promoting
545 phytohormones for their functions in promoting cell elongation and differentiation.

Defects in either of their biosynthesis or signaling transduction could result in inhibited plant growth and dwarfism (Schwechheimer, 2011; Fridman and Savaldi-Goldstein, 2013). The overlapping functions of BR and GA implied the potential crosstalk between them, which attracted extensive attentions in the recent one decade (Li and He, 2013). A breakthrough was the signaling interaction between these two hormones reported by three different laboratories at the same time. In this signaling model, the negative regulator of GA signaling, DELLAs, can physically interact with BES1 and BZR1, the positive regulators of BR signaling (Bai *et al.*, 2012; Gallego-Bartolomé *et al.*, 2012; Li *et al.*, 2012). Specifically, DELLAs only interacted with the dephosphorylated active BZR1 and further inhibited BZR1-DNA binding ability (Bai *et al.*, 2012). This DELLA-BES1/BZR1 interaction also existed in our research that tomato DELLA protein can physically bind to SIBES1.8 to interfere with its function by abolishing SIBES1.8-DNA binding (Fig. 7). Later, the synthesis model was proposed based on the results obtained from Arabidopsis and rice that BR regulated the biosynthesis of GA. In Arabidopsis, the expressions of multiple GA biosynthesis genes *GA20oxs* and *GA3oxs* were impaired in BR mutants. Correspondingly, the bioactive GA contents were reduced, which was partly responsible for the growth defects of BR mutants because external GA application or expression of *GA20ox1* partially restored the defects. Furthermore, the direct regulation of BES1 to the promoters of *GA20ox1*, *GA3ox1* and *GA3ox4* were verified (Unterholzner *et al.*, 2015). Similarly, BZR1 directly bound to the promoters of *GA20ox2*, *GA3ox2* and *GA2ox3* to regulate their expression in rice (Tong *et al.*, 2014). In our study, we determined the direct repression of SIBES1.8 to *SlGA2ox2* and *SlGA2ox6*, which led to the increase of bioactive GA contents that resulted in a similar phenotype to increased GA level or response. Taken together, our results obtained from tomato, in a certain extent, expanded our knowledge to BR and GA crosstalk both in signaling level and biosynthesis level, and more efforts need to be made in elaborating the crosstalk of these two hormones in different species.

As the key regulators of BR signaling, *BES1* genes involved in numerous biological

processes by directly interacting with key elements from other pathways. The functional identification of *BES1* family genes were widely investigated in Arabidopsis and other species, with critical roles in regulating cell elongation and division (Xie *et al.*, 2011), stress tolerance (Nolan *et al.*, 2017), immune signaling (Kang *et al.*, 2015), meristem differentiation (Kondo *et al.*, 2014) and male sterility (Chen *et al.*, 2019). Particularly, in tomato, the functional investigations of this family were mainly focused on the two key members, SIBES1 and SIBZR1. With convincing evidences, SIBES1 and SIBZR1 were verified to play important roles in regulating heat stress tolerance (Yin *et al.*, 2018), mediating autophagosome formation (Wang *et al.*, 2019), affecting pollen development (Yan *et al.*, 2020), influencing wood formation (Lee *et al.*, 2021), releasing apical dominance (Xia *et al.*, 2021) and promoting fruit softening (Liu *et al.*, 2021), while little is known about the functions of other members. Our findings that SIBES1.8 greatly influences tomato leaf morphogenesis obviously enriched the functional diversity of tomato *BES1* family, and more functional analyses of this family members in tomato and other species would be benefit to understand the crosstalk of BR with other pathways in regulating plant growth and stress responses.

592

593 **Supplementary data**

594 Supplementary data are available at *JXB* online.

595 **Fig. S1.** Relative mRNA level of *SIBES1.8* in over-expressed lines.

596 **Fig. S2.** Phenotype of one-month-old WT and OE-*SIBES1.8* plants in *Ailsa Craig*
597 background.

598 **Fig. S3.** Knockout of *SIBES1.8* didn't influence the normal development of tomato
599 leaf.

600 **Fig. S4.** Overexpression of *SIBES1.8* resulted in similar consequence as the
601 exogenous application of GA₃ in leaf morphogenesis.

602 **Fig. S5.** Expression pattern of genes involved in GA deactivation and signaling in WT
603 and OE-*SIBES1.8* leaves in response to GA₃ application.

604 **Fig. S6.** Amino acids alignment of tomato and Arabidopsis DELLA proteins by
605 ClustalX.

606 **Fig. S7.** The PEST domain of SIBES1.8 and DELLA motif of SIDEELLA are critical
607 for their interaction.

608 **Table S1.** Primers used in this study.

609 **Table S2.** GAs contents in WT and OE-*SIBES1.8* leaves.

610 **Table S3.** DEGs after GA₃ treatment in WT leaves.

611 **Table S4.** DEGs resulted by OE-*SIBES1.8*.

612 **Table S5.** 42 DEGs influenced by both GA₃ and OE-*SIBES1.8*.

613

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620

621 **Author contributions**

622 Z. L. supervised the research; Z. L., Z. X. and D. S. designed the experiments; D. S.,
623 W. X., Q. L. and L. W. performed the experiments; Y. L. provided experimental
624 assistance; D. S. wrote the manuscript and Z. X., Y. L. and Y. S. revised the paper. All
625 authors had read and approved the final manuscript.

626

627 **Conflicts of interest**

628 The authors declare no conflict of interest.

629

630 **Data availability**

631 All data supporting the results of this study are included in the paper and its
632 supplementary files.

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Figure legends

Fig. 1. Overexpression of *SIBES1.8* resulted in changed morphogenesis of tomato leaf. (A, B) The mature leaf at 35 DPG and leaf primordium at 15 DPG of WT (A) and OE-*SIBES1.8* (B) plants in micro-tom background. TL, terminal leaflet; LL, lateral leaflet; LLP, lateral leaflet primordium; Se, serration; DPG, days post germination; bar in mature leaf indicates 1 cm, bar in leaf primordium indicates 100 μ m. (C) Serration numbers of terminal leaflet and lateral leaflet in mature leaves. At least 20 leaves were counted for each column. (D) Leaves of wild type and OE-*SIBES1.8* plants in *Ailsa Craig* background. L, leaf; bar = 2 cm. (E) Silhouettes of the seventh leaf from wild type and OE-*SIBES1.8* plants (*Ailsa Craig* background) at 45 DPG. 1st indicates terminal and primary lateral leaflets; 2nd indicates secondary lateral leaflets; 3rd indicates intercalary leaflets; bar = 2 cm. (F) Leaflet numbers of wild type and OE-*SIBES1.8* plants presented in (D). At least 20 leaves were counted for each column. ** and *** refer to significant differences between WT and OE-*SIBES1.8* lines with $P < 0.01$ and $P < 0.001$ respectively (two-tailed Student's *t*-test).

Fig. 2. Overexpression of *SIBES1.8* resulted in similar consequence as the exogenous application of GA₃ in leaf morphogenesis. (A) Leaf phenotype of WT seedlings at 16 DPG without GA₃ application. (B) Leaf phenotype of WT seedlings at 16 DPG with 50 μ M GA₃ application. (C) Leaf phenotype of OE-*SIBES1.8* seedlings at 16 DPG without GA₃ application. Arrowhead indicates the serration of leaf. Blue and red circles indicate lateral leaflet (LL) and lateral leaflet primordium (LLP) respectively. L, leaf; white bar = 1cm; black bar = 1 mm.

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Fig. 4. Endogenous gibberellin (GA) contents in WT and OE-*SIBES1.8* leaves. (A) Part of generalized scheme of GA biosynthesis and deactivation in higher plants. Serial numbers indicate those GAs that have been detected in our study. 2ox, GA 2-oxidase; 3ox, GA 3-oxidase; 13ox, GA 13-oxidase; 20ox, GA 20-oxidase. (B) Contents of bioactive and inactive GAs detected in our study. (C) Total GAs contents in WT and OE-*SIBES1.8* leaves. (D) Proportion of two richest GAs (GA₃ and GA₈) in WT and OE-*SIBES1.8* leaves. (E) Proportion of another two bioactive GAs (GA₁ and GA₄) in WT and OE-*SIBES1.8* leaves. Values are means $\pm$ SD of three biological replicates. * refers to significant differences between WT and OE-*SIBES1.8* line with $P < 0.05$ (two-tailed Student's *t*-test).

Fig. 5. Transcriptome analysis of WT and OE-*SIBES1.8* leaves with or without GA₃ application. (A) Numbers of up- or down-regulated differentially expressed genes (DEGs, FC > 2, $Q < 0.05$) in distinct comparisons. (B) Venn diagram showing the overlap between sets of DEGs influenced by GA₃ treatment in WT (WT GA₃ / WT Mock) or by overexpression of *SIBES1.8* (OE Mock / WT Mock). (C) Heat map showing the expression profiles of 42 common DEGs in (B). Group □ containing DEGs that are both up- or down-regulated by GA₃ and OE-*SIBES1.8*. Group □ containing DEGs that are regulated by GA₃ and OE-*SIBES1.8* with opposite trends.

(D) Expression comparison of those 42 common DEGs after GA₃ application in WT and OE-*SIBES1.8* leaves. Group □ and □ containing DEGs with smaller or bigger effect respectively by GA₃ application in OE-*SIBES1.8* leaves compared with WT. FC, fold change.

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(A) Yeast two-hybrid (Y2H) assay for interaction between SIDE*LLA* and SIBES1.8 proteins. AD-T plus BD-53 or BD-Lam were used as the positive and negative control respectively. DDO, SD/-Leu/-Trp medium; QDO, SD/-Ade/-His/-Leu/-Trp medium. (B) Bimolecular fluorescence complementation (BiFC) assay in tobacco leaves for interaction between SIBES1.8 and SIDE*LLA* proteins. Bar = 50 μm. (C) Firefly luciferase complementation imaging (LCI) assay in tobacco leaves for interaction between SIBES1.8 and SIDE*LLA* proteins. (D) Diagram of reporter and effectors used in dual-luciferase assays. SIDE*LLA* and SIBES1.8 were fused with GAL4BD to generate the effector constructs. VP16 was used as the transcriptional activation control. (E) Suppression of SIDE*LLA* on the transcriptional repression activity of SIBES1.8 detecting by GAL4-responsive reporter system in tobacco leaves. Reporter was co-expressed with SIBES1.8 or SIDE*LLA* effector alone or together in tobacco leaves. The LUC/REN ratio of the group of empty effector was regarded as calibrator (set as 1). At least six biological replicates were used for each column. The significant differences are indicated with letters (*P* < 0.05, one-way ANOVA test). (F) EMSA showing the inhibition of SIDE*LLA* on the binding of SIBES1.8 to the promoters of *SIGA2ox2*, *SIGA2ox6* and *SIGID1b-1*. Biotin probes were incubated with GST-SIBES1.8 alone or together with GST-SIDE*LLA* protein. ++ indicates increased amount of GST-SIDE*LLA* proteins; m, mutant biotin probe.

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