RESEARCH PAPER

Tomato *Sidreb* gene restricts leaf expansion and internode elongation by downregulating key genes for gibberellin biosynthesis

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Abstract

Plants have evolved and adapted to different environments. Dwarfism is an adaptive trait of plants that helps them avoid high-energy costs under unfavourable conditions. The role of gibberellin (GA) in plant development has been well established. Several plant dehyhydration-responsive element-binding proteins (DREBs) have been identified and reported to be induced under abiotic and biotic stress conditions. A tomato DREB gene named *Sidreb*, which is a transcription factor and was cloned from cultivated tomato M82, was found to play a negative role in tomato plant architecture and enhances drought tolerance. Tissue expression profiles indicated that *Sidreb* was expressed mainly in the stem and leaf and could be induced by abscisic acid (ABA) but suppressed by GA and ethylene. *Sidreb* altered plant morphology by restricting leaf expansion and internode elongation when overexpressed, and the resulting dwarfism of tomato plants could be recovered by application of exogenous gibberellic acid (GA 3 ). Transcriptional analysis of transgenic plants revealed that overexpression of *Sidreb* caused the dwarf phenotype by downregulating key genes involved in GA biosynthesis such as *ent*-copalyl diphosphate synthase (*ScPS*) and GA 20-oxidases (*GaA20ox1*, -2, and -4), thereby decreasing endogenous GA levels in transgenic plants. A yeast activity assay demonstrated that *Sidreb* specifically bound to dehydration-responsive element/C-repeat (DRE/CRT) elements of the *ScPS* promoter region. Taken together, these data demonstrated that *Sidreb* can downregulate the expression of key genes required for GA biosynthesis and that it acts as a positive regulator in drought stress responses by restricting leaf expansion and internode elongation.

Key words: DREB, dwarfism, *ent*-copalyl diphosphate synthase, gibberellin, gibberellin 20-oxidase, tomato.

Introduction

Drought, high salinity, and low temperature are adverse environmental conditions that affect plant distribution (Yang et al., 2011). When plants encounter adverse ambiance, they exhibit a variety of responses that result in specific changes suited to the particular stress condition encountered. For example, the dwarf phenotype benefits the plant through avoidance of the high-energy cost of producing stress-tolerance proteins (Knight and Knight, 2001). The endogenous phytohormone gibberellin (GA) is involved in plant growth and development including internode elongation, and is one of the compounds associated with the dwarf phenotype (Hedden and Kamiya, 1997). GA biosynthesis and catabolism...
genes in higher plants have been well characterized. Geranyl diphosphate synthase (GPS) has been reported to control the level of bioactive GA by modulating GA precursor biosynthesis in tomato (van Schie et al., 2007). Geranyldgeranyl diphosphate (GGDP) is the common precursor for diterpenoids including GAs, and is converted to ent-kaurene through successive two cyclization reaction catalyzed by ent-copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS). The ent-kaurene is then converted to GA2 by the two distinct P450 monoxygenases, ent-kaurene oxidase (KO) and ent-kaurenocixidase (KAO). GA12 can be further converted to GA2 by 13-hydroxylation. GA12 and GA45 are converted mainly to various GA intermediates (e.g. GA4 and GA30) and successively to bioactive GAs (e.g. GA3 and GA4) by GA 20-oxidases (GA20ox) and GA 3-oxidases (GA3ox), respectively. GA3ox catalyses the final step in the synthesis of bioactive GAs. GA 2-oxidase (GA2ox) is the major enzyme that converts active GAs (GA2, and GA4) and their precursors (GA9 and GA30) to inactive forms (Yamaguchi, 2008; Sun, 2011).

Over the past decade, a family of transcription factors known as APETALA2 (AP2)/ethylene-responsive element binding factor (ERF) has been identified in plants. These proteins are involved in a variety of regulatory mechanisms such as fruit ripening (Chung et al., 2010), growth and development (Wilson et al., 1996), and biotic (Thara et al., 1999; Brown et al., 2003) and abiotic stresses (Liu et al., 1998; Sakuma et al., 2002; Fukao and Bailey-Serres, 2008; Li et al., 2011; Wan et al., 2011; Zhang et al., 2011; Shi et al., 2012). The AP2/ERF transcription factors are found only in the plant kingdom and are characterized by the presence of a highly conserved DNA-binding domain (Riechmann and Meyerowitz, 1998; Tournier et al., 2003). ERFs are part of the AP2/ERF superfamily, which also contains AP2 and RAV family genes and is characterized by the presence of the AP2/ERF DNA-binding domain (Sakuma et al., 2002). Based on their ERF domain, the tomato ERFs can be further divided into two subfamilies, ERF and DREB (dehydration-responsive element-binding proteins), and their ERF domain diversity is manifested at amino acid positions 14 and 19 (Sharma et al., 2010). DREBs contain a conserved AP2/ERF domain that binds specifically to the dehydration-responsive element/C-repeat (DRE/CRT) cis-acting element (core motif: G/ACCGAC) of the genes that they regulate (Busk et al., 1997; Qin et al., 2004).

DREB-binding factors play crucial roles in the regulation of abiotic and biotic responses in plants. Heterogeneous expression of a DREB gene from Leymus chinenesis improved drought and salt tolerance of Arabidopsis thaliana (Xianjun et al., 2011). Similarly, expression of a DREB gene from Limonium bicolor enhanced copper tolerance in transgenic tobacco plants (Ban et al., 2011). A rice DREB gene, ARAG1, which is involved in the abscisic acid (ABA) signalling pathway, plays a role in seed germination and drought tolerance (Zhao et al., 2010). Arabidopsis DREBs play a role in the regulation of water homeostasis by regulating multiple aquaporin genes (Rae et al., 2011).

The Arabidopsis DREB protein DEAR1 has an upstream regulatory role in mediating cross-talk between signalling pathways for biotic and abiotic stress responses (Tsutsui et al., 2009). Moreover, two chrysanthemum DREB genes have been reported to play a regulatory role in abiotic stress responses (Yang et al., 2009), and overexpression of OsDREB1G and OsDREB2B genes in rice significantly improved their tolerance to water deficit stress (Chen et al., 2008).

Here, we demonstrate that SIDREB encodes a transcription factor whose expression is regulated by drought, salt, and cold, and by ABA, gibberellic acid (GA3), and ethylene treatments. Our experimental results revealed that overexpression of SIDREB in tomato led to lower endogenous GA levels and consequently a dwarf phenotype consisting of restricted leaf expansion and internode elongation via downregulation of key genes involved in GA biosynthesis. Notably, SIDREB could bind specifically to the DRE/CRT elements of the SICPS promoter region, indicating that SIDREB may directly regulate the expression of SICPS in tomato.

Materials and methods

Plant material and plant growth regulator treatment

Tomato (Solanum lycopersicum) variety M82 and wild species Solanum pennellii (LA0716) were used to compare SIDREB nucleotide sequences between wild and cultivated species. S. lycopersicum cv. M82 was used to analyse the expression of SIDREB in different organs and the response to different plant growth regulators. Tissues from the roots, stems, leaves, flowers, and fruits of M82 plants were collected and immediately frozen in liquid nitrogen and stored at −80 °C until use. For plant growth regulator treatment, four-leaf-stage plants grown in compost plastic trays in a 16 h light/8 h dark regime at 25 °C in a greenhouse were sprayed with 100 µM ABA, 100 µM GA3, 100 µM ethephon (Eth; an ethylene releaser), or distilled water (control). Three leaves, one from each plant, were collected at the designated times from the different treated and untreated plants and stored as indicated above. Plant morphology was assessed by measuring the internode lengths and number of leaves, and leaf samples were taken 35 d post-inoculation (p.i.). Dwarf phenotypes were rescued by spraying with a 100 µM GA3 solution containing 0.02% Tween 20 at an interval of 3 d until run-off starting from 30 d p.i.

Isolation of SIDREB, construction of overexpression vectors, and plant transformation

In our previous studies on drought stress in tomato introgression lines (ILs), a differential expression profile of the SIDREB gene was observed between the drought-tolerant ILs and M82 (Gong et al., 2010). The tomato SIDREB gene was PCR amplified from the cDNA of M82 (forward primer: 5'-CCAATCTCTCTCCCTAAA-3'; reverse primer: 5'-TTTCAATGTTTACCAATGTTCT-3') based on the UniGene sequence (SGN-U585938). For transformation, we used the binary plasmid vector pMV2 (Yang et al., 2011a), which carries the spectinomycin resistance gene for bacterial selection and the neomycin phosphotransferase II gene for selection of transformed plants. The binary plasmid was constructed by inserting the SIDREB cDNA between the KpnI and XbaI sites in the sense orientation driven by the cauliflower mosaic virus 35S promoter. The plasmid mediated by Agrobacterium tumefaciens strain C58 was transformed in tomato cultivar M82. After screening for regenerated shoots on selection medium containing kanamycin, the transgenic plants were further verified by PCR using genomic DNA as template and 35S forward and gene-specific reverse primers.

RNA isolation and real-time RT-PCR

Total RNA was isolated using TRIzol reagent (Invitrogen, USA). DNase I (Fermentas, USA)-treated RNA was reverse transcribed using a Moloney murine leukaemia virus cDNA reverse transcription enzyme (Invitrogen) and cDNA was used for real-time RT-PCR. Real-time
RT-PCR was performed with a SYBR Premix Ex Taq kit (Takara, Japan) using primers (see Supplementary Table S1 at JXB online) specific for *SIDREB*, *SIGPS* (DQ86930), *SICPS* (AB015675), *SIK* (AB28778), *SIKO* (SGN-US83328), *SIKAO* (SGN-US575348), *SIGA2ox1* (AF049898), *SIGA2ox2* (AF049899), *SIGA2ox3* (AF049900), *SIGA2ox4* (EU652334), *SIGA3ox1* (AB100991), *SIGA3ox2* (AB100992), *SIGA2ox1* (EF441351), *SIGA2ox2* (EF441352), *SIGA2ox3* (EF441353), *SIGA2ox4* (EF441354), *SIGA2ox5* (EF441355), *SIGASTI* (X65083), *SIDELLA* (AY269087), and β-actin (SGN-US86009) transscripts as internal controls. The PCR amplification step consisted of an initial incubation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s. Data were collected during the extension step, and melting-curve acquisitions and analyses were also performed on the cycler. PCR products were monitored using a LightCycler 480 (Roche, Switzerland) PCR system.

Subcellular localization assay of *SIDREB*

The full-length open reading frame (ORF) without the stop codon of *SIDREB* was PCR amplified using primers containing KpnⅠ and BamHI restriction sites (underlined) (forward: 5′-GGTACCTGCTCAA AGCGAATAGAGGAGTG-3′; reverse: 5′-GGATCTTTTCTCATG TTCAAGGTCTGTAAG-3′). The PCR product was purified from agarose gel, cloned into a pMD18-T vector (TaKaRa), and sequenced. The plasmid containing the correct sequence of *SIDREB* was digested with KpnⅠ and BamHI and subcloned into a pCAMBIA1391 vector digested using the same restriction enzymes to create a fusion construct (pCAMBIA1391-SIDREB-GFP). Both the fusion construct (pCAMBIA1391-SIDREB-GFP) and the control vector (pCAMBIA1391-GFP) were transformed into Y1H Gold yeast strain. Selection for transformants was done on SD/–Trp or SD/–Ura medium. After PCR confirmation, the second primer (200 ng ml−1) was added as internal standards: [1H2]GAI (1.00 ng g−1), [1H2]GAl (1.00 ng g−1), [1H2]GA3 (2.00 ng g−1), [1H2]GA4 (2.00 ng g−1), [2H2]GA53 (4.00 ng g−1).

Quantification of endogenous GAs

The leaves of tomato (1 g) were frozen in liquid nitrogen and finely ground follow by extraction with 15 ml of methanol containing 20% water (v/v) at 4 °C for 12 h. Before grinding, the following labelled GAs were added as internal standards: [1H2]GAI (1.00 ng g−1), [1H2]GAl (1.00 ng g−1), [1H2]GA3 (2.00 ng g−1), [1H2]GA4 (2.00 ng g−1), [1H2]GA5 (2.00 ng g−1), and [1H2]GA53 (4.00 ng g−1). Further sample was prepared and analysed as described previously (Chen et al., 2011). In brief, the sample was first passed through a C-18 SPE cartridge (12 ml, 1.5 g), and the resulting eluate was evaporated under a nitrogen gas stream and redissolved in 3 ml of water. The solution was acidified with 360 µl of 0.1 M hydrochloric acid and extracted repeatedly with ethyl ether (10 × 0.5 ml). The ether phases were combined, dried under nitrogen gas, and redissolved in 112 µl of acetonitrile. After the addition of 180 µl of Et3N (20 µmol ml−1) and 108 µl of 3-bromoacetylimidazolium bromide (20 µmol ml−1), the reaction solution was vortexed for 10 min. The mixture was evaporated under nitrogen gas and the residue was dissolved in 30 µl of water. The prepared samples were injected using 25 kV×1 min and separated by 100 cm amino groups, and coated capillary electrophoresis coupled with electrospray ionization quadrupole-time-of-flight mass spectrometry was performed for analysis.

Scanning electron microscopy for leaf thickness and stomatal observation

For scanning electron microscopy observation, the third leaves from *SIDREB* was bound to DRE/CRE of the *SICPS* promoter region. We isolated the ~2500 putative promoter regions of *SICPS* from the SGN database (http://solgenomics.net/) and confirmed their sequence. The full-length *SIDREB* was fused to the GAL4 activation domain in the vector pGADT7 digested with *Smal* and *BamHI* to get pGAD-SIDREB. A 48 bp single-stranded oligonucleotide sequence (5′-AGTACCTGACTCCGACTCATCCGACTGACACTGGCATTC-3′) and its reverse complement sequence (5′-TGCGAGGTGATGAGTGGGTAATCAGGAGCTGCTA-3′), which contains three tandem repeat copies of 5′-CATG TACGACTCC-3′ (DRE/CRT is underlined) in the *SICPS* promoter regions and cohesin terminal of *HindIII* and *XhoI* was synthesized. The oligonucleotides were annealed as follows: each single-stranded oligonucleotide of 100 µM mixes at a ratio of 1:1, yielding a final concentration of 50 µM each, were heated to 95 °C for 30 s, 72 °C for 2 min, 37 °C for 2 min and 25 °C for 2 min. After annealing, the double-stranded oligonucleotides were cloned into a pAbAI vector linearized using the restriction enzymes *HindIII* and *XhoI*, and their sequences were confirmed. The resulting pDRE-AbAi construct and p53-AbAi control vector were digested with *BsrBI*, and the linearized plasmids were transformed into Y1H Gold yeast strain. Selection for transformants was performed on SD–Ura medium. After PCR confirmation, the minimal inhibitory concentration of aureobasidin A (AbA) for the pDRE-AbAi yeast strain was determined. The p53-AbAi control had a minimal inhibitory concentration of 100 ng ml−1 AbA (Clontech). The pGAD-SIDREB and pGADT7 vectors were transformed into the pDRE-AbAi yeast strain, while the pGAD-p53 vector was transformed into p53-AbAi containing Y1H Gold yeast strain as a positive control. A large healthy colony was picked from the yeast strains and suspended in 0.9% NaCl. The optical density at 600 nm was adjusted to 0.002 (for ~2000 cells per 100 µl), and 2 µl of cells was also dotted on the SD–Ura–Leu medium with or without AbA to assess DNA–protein interactions. The colonies were then allowed to grow for 2–3 d at 30 °C.

Results

**Tomato SIDREB is a typical transcription factor**

The AP2/ERF superfamily is defined by the AP2/ERF domain, which consists of ~60–70 amino acids and is involved in DNA binding. There are 112 AP2/ERFs in tomato, 85 of which are potential ERFs with a single complete AP2/ERF domain. The ERFs have been divided further into two subfamilies, ERF and DREB (Sharma et al., 2010). Based on a phylogenetic analysis based of all tomato ERFs, SIDREB was classified into the DREB subfamily (Fig. 1).

The tomato DREB gene was previously identified as a drought-responsive gene in our microarray experiment (Gong et al., 2010). We confirmed that it is induced by drought, salt, and...
Using PCR, we isolated and cloned the full-length (579 bp) ORF of SlDREB from the cDNA of *S. lycopersicum* cv. M82 and of SpDREB from *S. pennellii*. Comparison of their deduced protein sequences revealed that SlDREB shared 97% similarity with SpDREB (from wild tomato) and 60% similarity with StDREB (from *Solanum tuberosum*) but only 53% similarity with AtDREB (from *Arabidopsis*) (Fig. S2A at JXB online). A difference of only three amino acids—threonine, serine, and glutamic acid (in M82) in place of serine, arginine, and lysine (in *S. pennellii*) at positions 110, 134, and 144, respectively (Fig. S2B)—was observed between SIDREB and SpDREB.

Bioinformatics analysis revealed a nuclear localization signal (positions 36–42) in the basic region of the AP2/ERF DNA-binding domain, implying that SIDREB may be localized in the nucleus. In order to verify our in silico result, subcellular localization of SlDREB was examined by monitoring GFP fluorescence in onion epidermis cells transformed with either a fusion construct (pCAMBIA1391-SIDREB-GFP) or a control construct (pCAMBIA1391-GFP). Green fluorescence signals were observed over the entire cell in onion cells transformed with the control construct (Fig. 2A–C). In contrast, fluorescence was exclusively detected in the nuclei of cells transformed with the fusion plasmid (Fig. 2D–H), implying that SIDREB is a nuclear protein.
In addition to nuclear localization, transactivation activity is another defining feature of a transcription factor. We used a yeast two-hybrid system to examine the transcriptional activity of SlDREB. A GAL4 DNA-binding domain SlDREB fusion protein was expressed in yeast cells, which were then assayed for their ability to activate transcription from the GAL4 sequence. SlDREB promoted yeast growth in the absence of histidine and adenine, and showed X-α-gal activity, whilst the vector control pGBKT7 did not (Fig. 3). These data confirmed that SlDREB functions as a transcriptional activator in yeast.

**Fig. 2.** Subcellular localization of SlDREB in onion epidermal cells. GFP and SlDREB–GFP fused constructs were expressed transiently in onion epidermal cells. Bright-field images (A, D), GFP fluorescent images (B, E), DAPI image (F), and merged images (C, H) of representative cells transformed with GFP (A–C) or the SlDREB–GFP fusion protein (D–G) are shown. (This figure is available in colour at JXB online.)

To investigate the function of SlDREB, the plasmid 35S:SlDREB was introduced into cultivated tomato M82. Transgenic plants overexpressing (OE) SlDREB were obtained after screening for regenerated shoots on selection medium containing kanamycin. The transgenic plants were analysed further by PCR using genomic DNA as template and 35S forward and gene-specific reverse primers. Twenty-three transformants (T0) regenerated from kanamycin-resistant calli contained SlDREB.

The expression level of the SlDREB gene in transgenic (T0, T1, and T2) as well as control plants was examined at the five-leaf stage by real-time PCR analyses. Expression of SlDREB was 42.84- and 19.33-fold greater in the overexpressing T2 homozygous line 4 (OE4) and overexpressing line 13 (OE13), respectively, compared with M82 control plants (Fig. 5E).

**Fig. 3.** Analysis of the transactivation activity of SlDREB. SlDREB and GAL4 DNA-binding domain fusion protein were expressed in the yeast strain AH109. Vectors pGBK7T7 and pGBK7T7-53+pGADT7-RecT were expressed in yeast as a negative and positive control, respectively. The yeast streak was cultured on SD/–Trp and SD/–Ade/–His/–Trp medium; both contained X-α-gal for assaying another yeast reporter (MEL1) gene. (This figure is available in colour at JXB online.)

**SIDREB is induced by GA3 and Eth but suppressed by ABA**

Real time-PCR detection results showed that SIDREB was highly expressed in tomato stems and leaves (Fig. 4A). By comparing the SIDREB expression levels of treated plants relative to those of untreated plants, we found that SIDREB expression was significantly suppressed by GA3 and Eth treatment for up to 6 h after treatment but returned to pre-treatment levels 12 h later. However, under ABA stress, SIDREB expression was induced from 1 to 6 h after treatment and returned to pre-treatment levels 12 h later (Fig. 4B, 4C, 4D). These expression patterns indicate that SIDREB was downregulated by GA3 and Eth treatment but upregulated by ABA application.

**Transgenic plants overexpressing SIDREB have shorter internodes and increased drought tolerance**

Transgenic plants overexpressing SlDREB have shorter internodes. For further experiments, we selected two T2 homozygous lines: OE4 and OE13 (eight individual plants of each line). The transgenic plants and WT plants were cultivated under the same conditions, and plant height was determined after 2 months. The average plant height of the control, OE4, and OE13 plants was 29.7, 11.3, and...
The mean number of stomata on functional leaves of \textit{SIDREB}-overexpressing plants was approximately seven compared with nine per unit area (~0.065 mm$^2$) in WT plants (Fig. 5H). This could reflect specific effects of \textit{SIDREB} on tomato architecture leading to lowered transpiration and energy loss under drought stress. Consistent with this idea, the \textit{SIDREB}-overexpressing plants performed very well, whilst the WT plants showed signs of wilting (Fig. 5G).

\textbf{Overexpression of \textit{SIDREB} downregulates GA biosynthesis genes}

To investigate whether \textit{SIDREB} altered GA metabolism, expression of GA biosynthetic genes was compared between \textit{SIDREB} transgenic and M82 (control) seedlings. Two genes in the GA biosynthetic pathway, \textit{SlCPS} and \textit{SlKS}, showed considerable change in expression in transgenic plants. \textit{SlCPS} was dramatically downregulated in \textit{SIDREB} transgenic seedlings: OE4 exhibited a 3.44-fold decline in \textit{SlCPS} expression compared with M82 (WT) seedlings. In contrast, expression of \textit{SlKS} was 2.18-fold greater in \textit{SIDREB} transgenic seedlings than in M82 WT seedlings (Fig. 7A, 7C). No significant difference in expression
Fig. 5. Overexpression of SIDREB restricts tomato leaf expansion and internode elongation. (A) Phenotypic appearance of seedlings (upper) and leaves (lower) of SIDREB-overexpressing tomato OE4 and OE13 lines and WT plants. (B) Leaflet size of WT and SIDREB lines OE4 and OE13. The third leaf from the top of 2-month-old transgenic and non-transgenic plants was used for leaflet size measurement; the apical leaflet was chosen from the leaf. Error bar indicates SE (n=8). (C) Internode length of 2-month-old WT and SIDREB OE4 tomato plants. Error bars indicates SE (n=8). (D) Scanning electron microscopy images showing leaf thickness. Leaves were collected from 2-month-old tomato SIDREB-overexpressing and WT plants grown in a naturally illuminated glasshouse. The apical leaflet of the third leaf from the top was collected for analysis. (E) The expression level of SIDREB in transgenic and WT control plants was examined at the five-leaf stage by real-time PCR analyses. Error bars indicates SE (n=8). (F) Graphical representation of leaf thickness. Eight individual plants were selected per line and the thickness of every leaf was measured three times. Asterisks indicate significant differences compared with WT (**P < 0.01). (G) SIDREB-overexpressing plants show drought resistance. At 40 d post-inoculation, watering was stopped for drought treatment. The photographs were taken after 15 d. Treatment was replicated three times under the same conditions (n > 8). (H) The mean number of stomata per unit area in SIDREB overexpressing and WT leaves. Stomatal number was counted in each field of view (400×, ~0.065 mm²) on at least 20 microscopes and three plants. The third leaf from the top of 2-month-old transgenic and non-transgenic plants was analysed. (This figure is available in colour at JXB online.)
of other genes responsible for the early steps of GA biosynthesis, such as *SlGPS*, *SlKO*, and *SlKAO*, was seen between *SlDREB* transgenic and WT plants (Fig. 7B).

GA20oxs are also key GA biosynthetic enzymes that determine GA concentration in many plant species, and GA3oxs catalyse the final step to produce bioactive GAs (GA1, GA3, and GA4) (Hedden and Kamiya, 1997; Xiao *et al.*, 2006; Yamaguchi, 2008). We compared transcript levels of GA20oxs and GA3oxs between transgenic plants and WT plants by quantitative RT-PCR. In transgenic plants, *SlGA20ox1*, *SlGA20ox2*, and *SlGA20ox4* were sharply downregulated, but *SlGA3ox1* and *SlGA3ox2* were slightly upregulated when compared with their respective expression in WT plants (Fig. 7A, 7C).

To determine whether *SlDREB* is involved in GA inactivation, transcript levels of genes encoding GA2oxs (the main GA catabolic enzyme) in tomato (i.e. *SlGA2ox1*, *SlGA2ox2*, *SlGA2ox3*, *SlGA2ox4*, and *SlGA2ox5*; Serrani *et al.*, 2008) were quantified by RT-PCR. No significant differences in expression of these genes were seen between transgenic and WT plants (Fig. 7B).

To determine further whether *SlDREB* affects GA signal transduction factors, the expression levels of *SIDELLA*, which has been reported to be a GA repressor in the GA response pathway (Sun and Gubler, 2004), and *SIGAST1*, a GA-responsive gene (Shi *et al.*, 1992), were compared by quantitative RT-PCR. Expression levels of *SIDELLA* and *SIGAST1* were not significantly different between the transgenic and WT plants (Fig. 7B).

**Decreased endogenous GA abundance in *SlDREB* overexpressing tomato plants**

As our results showed that *SlDREB* repressed the expression of GA biosynthesis genes, we decided to compare endogenous GA levels in *SlDREB* overexpressing transgenic and WT plants. As shown in the schematic representation of GA biosynthesis in Fig. 7C, endogenous levels of several GAs (GA53, GA12, GA3, GA20, GA4, and GA1) in *SlDREB*-overexpressing transgenic plants were significantly lower than in WT plants.

**SIDREB specifically binds to the DRE/CRT elements of the SlCPS promoter region.**

Previous studies have shown that DREBs can bind to the *cis* element of DRE/CRT (Busk *et al.*, 1997; Qin *et al.*, 2004). We found that the promoter region (~1928) of SlCPS contains a DRE/CRT element (Fig. S3 at JXB online). In order to investigate whether *SlDREB* binds to the DRE/CRT elements of the promoter region of the SlCPS gene, the full-length ORF of *SIDREB* was fused to the GAL4 activation domain of the vector pGADT7. The fused construct thus obtained (pGADT7-SIDREB) was co-transformed with the pDRE-AbAi construct containing triple tandem repeats of DRE/CRT from the SlCPS promoter region into the Y1H Gold yeast strain (Fig. 8). Although all yeast cells harbouring different constructs could grow on SD/-Ura without AbAi, those

![Fig. 6. The dwarf phenotype of *SlDREB*-overexpressing plants was rescued by spraying with GA3. (A) Pictures were taken 50 d after inoculation. The dwarf phenotype was rescued by spraying GA3 every 3 d starting from 30 d after inoculation. (B) Plant height is displayed as the mean of eight plants; error bars show SE (n=8). (This figure is available in colour at JXB online.)](image-url)
Fig. 7. Decreased GA abundance and GA biosynthesis gene expression in SIDREB-overexpressing tomato plants. (A, B) Real-time RT-PCR analysis of the expression of tomato GA biosynthesis genes in OE4, OE13 and WT plants. *P < 0.05; **P < 0.01. Primer sequences are listed in Supplementary Table S1. All samples were collected at the indicated time points from three biological replicates in each treatment group. Error bar indicates the SE of three replicates. (C) Pathway of GA biosynthesis: green indicates significantly (P < 0.05) downregulated expression and red indicates slightly upregulated expression. Numbers indicate endogenous GA levels in the WT (upper) and transgenic plants (lower, boxed). Results that were ‘not detected’ were due to low abundance; **P < 0.01; * P < 0.05. Data are presented as means ±standard deviation from three technical replications (ng g⁻¹ of fresh weight) using two transgenic lines. IPP, prenyldiphosphates isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPS, geranyl diphosphate synthase; GGDP, geranylgeranyl diphosphate; CPD, copalyl diphosphate; CPS, ent-copalyl diphosphate synthase; KS, ent-kaurene synthase, KO, ent-kaurene oxidase; KAO, ent-kaurenoic acid oxidase. (This figure is available in colour at JXB online.)
with pDRE-AbAi did not grow in the presence of 40 ng ml\(^{-1}\) of AbA (Fig. 8, upper panel). However, cells co-transformed with pGADT7-SlDREB and pDRE-AbAi grew normally in the presence of the minimal inhibitory concentration (40 ng ml\(^{-1}\)) of AbA. Additionally, the growth of transformants containing constructs lacking SlDREB was completely inhibited (Fig. 8, lower panel), suggesting that SlDREB could bind to the DRE/CRT cis element in yeast.

**Discussion**

**SIDREB is an AP2/ERF-binding factor and is regulated by GA, ABA, and Eth in tomato**

AP2/ERF proteins have been shown to be integrators of biotic and abiotic stress responses through their interaction with cis-acting elements, the GCC box, and CRT/DRE (Knight and Knight, 2001; Sakuma et al., 2002; Brown et al., 2003; Li et al., 2011). DREB can be classified into the ERF subgroup of the AP2/ERF superfamily based on its function and group motif (Sharma et al., 2010). DREB transcription factors are involved in abiotic and biotic stress signalling pathways in plants (Agarwal et al., 2006). However, the functions of DREB in tomato remain unclear. **SIDREB** was isolated by screening the results of our previous microarray experiments on drought-tolerant tomato (Gong et al., 2010). **SIDREB** was responsive to drought, salt, and cold stresses and could be classified into subgroup III of the isolated DREBs (Figs 1 and S1). In addition, it was also shown that SIDREB is localized in the nucleus and exhibits transactivation activity in yeast (Figs 2 and 3), suggesting that **SIDREB** encodes a typical transcriptional factor.

It has been reported that AP2/ERF transcription factors can modulate ethylene responses (Chang and Shockey, 1999; Yang et al., 2011). Ethylene has been found to promote GA responsiveness and shoot elongation in submergence-intolerant lines and to confer submergence tolerance in rice (Fukao and Bailey-Serres, 2008). Moreover, internode elongation of rice in deepwater requires the activity of GA and ethylene (Sauter et al., 1995). Under deepwater conditions, ethylene accumulates and triggers remarkable internode elongation via GAs (Hattori et al., 2009). It seems that GA\(_3\) and Eth provide collaboration features in the process of plant internode elongation. This is consistent with our observation that **SIDREB** had the same expression profile under GA\(_3\) and Eth treatment (Fig. 4B, 4C), and it is possible that **SIDREB** can mediate cross-talk between ethylene and GA in plant internode elongation.

GAs regulate various developmental processes including stem elongation, leaf expansion, fruit development, and seed germination (Dill et al., 2004). GA and ABA play antagonistic roles in the regulation of numerous developmental processes: GA is associated with the promotion of germination, growth, and flowering, whilst ABA acts as a competitive inhibitor of GA activity.
and inhibits these processes (Razem et al., 2006). This antagonistic relationship was partially confirmed by our observation that expression of SIDREB was upregulated by GA application and downregulated by ABA treatment (Fig. 4B, 4D). It appears that SIDREB functions as a balance regulator through different networking between GA and ABA signalling pathways.

SIDREB restricts leaf expansion and internode elongation by negative regulation of GA biosynthesis genes

Our results demonstrated that the dwarf phenotype of SIDREB-overexpressing plants could be attributed to decreased levels of endogenous GAs (Fig. 7C). Accordingly, dwarfism of SIDREB-overexpressing plants could be reversed by the application of GA3 (Fig. 6A, 6B). These results are in agreement with previous studies in GA-deficient mutants of various plants, including Zea mays (Fujioha et al., 1988), Arabidopsis thaliana (Magome et al., 2004), and Oryza sativa (Sakamoto et al., 2004).

To assess further whether the GA-sensitive dwarf phenotype of SIDREB-overexpressing plants was due to inhibition of transcription of GA biosynthetic genes, inhibition of GA signal transduction, and/or promotion of expression of GA-inactivating enzyme genes, we analysed the expression level of different genes involved in these pathways. Our results demonstrated that SIDREB restricted internode elongation and leaf expansion by downregulating the expression of SICPS and SIGA20ox1, -2, and -4 (Fig. 7A), which encode key enzymes in the GA biosynthesis pathway. In fact, a series of examples showed that CPS and GA20ox genes were targeted to lead to alteration of GA level and a dwarf plant. Loss-of-function CPS mutants of several plant species, such as rice (Sakamoto et al., 2004), Arabidopsis thaliana (Sun and Kamiya, 1994), and Pisum sativum (Ait-Ali et al., 1997), have also been shown to display severe dwarf phenotypes. In contrast, Serrani et al. (2008) reported that upregulation of SICPS, SIGA20oxs, and SIGA3ox by 2, 4-dichlorophenoxyacetic acid treatment led to higher levels of bioactive GA in tomato. GA20ox catalyses the sequential oxidation of C-20 and is responsible for the production of inactive precursors (GA9, GA20) during bioactive GA synthesis (Yamaguchi, 2008). Overexpression and downregulation of GA20ox modified plant height by altering the levels of active GAs (Coles et al., 1999; Carrera et al., 2000; Vidal et al., 2001; Fagoaga et al., 2007). No significant differences were found in the expression of other genes involved in GA biosynthesis (SIGPS, Sik0, SikAO, SIGA20ox3, and SIGA3ox1 and -2), and the GA-inactivating enzyme (SIGA2ox1–5) between SIDREB-overexpressing lines and WT plants (Fig. 7B). On the other hand, expression of SIGA20ox3 and SikS was slightly upregulated in transgenic plants, probably for the feedback of GA deficiency (Cowling et al., 1998). Taken together, our results show that SIDREB restricts internodal elongation and leaf expansion by downregulating the expression of key GA biosynthesis enzymes. Moreover, the expression of GA response genes (SIdEL1 and SIGA3T1) between SIDREB-overexpressing and WT plants was not significantly changed. This provides further evidence that SIDREB negatively regulates the internode elongation and leaf expansion processes by inhibiting GA biosynthesis and not by signal transduction.

SIDREB may directly regulate expression of SICPS

Transcription factors bind to either enhancer or promoter regions of DNA to regulate the expression of genes (Latchman, 1997). Most transcription factors act either as activators or repressors (Ikeda et al., 2009); therefore, depending on the transcription factor, the transcription of the adjacent gene is either up- or downregulated (Gill, 2001). Transcriptional regulation is an important mechanism for controlling metabolic pathways. These pathways are generally influenced by environmental stimuli and the plant’s developmental programme (Van Moerkercke et al., 2011). As we found that expression of SICPS and SIGA20ox1, -2, and -4 was negatively related to SIDREB expression in our study (Fig. 7A), we studied the binding of SIDREB with the promoter region of genes it is likely to regulate. Our results showed binding of SIDREB to the DRE/CRT domains of the SICPS promoter region (Fig. 8), and thus reveal that SIDREB likely

![Diagram](Image 340x181 to 580x546)

**Fig. 9.** Model of SIDREB regulation of leaf expansion and internode elongation by downregulating key genes required for gibberellin biosynthesis. SIDREB directly or indirectly downregulates the expression of SICPS and SIGA20oxs, respectively. Transgenic plants overexpressing SIDREB have lower endogenous GA levels, leading to restriction of leaf expansion and internode elongation, which in turn increases the drought tolerance of plants.
acts as a direct repressor of \textit{SICPS} in plants. We did not find DRE/CRT elements in the putative 2500bp promoter regions of \textit{SIGA20ox1}, -2, and -4, which might have been downregulated in the \textit{SIDREB}-overexpressing plants through other mechanisms.

\textbf{SIDREB integrates environmental stress signals and reduces endogenous GA levels to avoid abiotic stress}

To sum up, overexpression of \textit{SIDREB} in tomato plants can downregulate the expression of \textit{SICPS} and \textit{SIGA20oxes}, and lowers endogenous GA levels, leading to restriction in leaf expansion and internode elongation. These effects account for the dwarfishm of tomato plants and help in drought tolerance (Fig. 9). It seems that, under stress, decreased GA levels inhibit growth, allowing plants to survive better in unfavourable conditions. This hypothesis was confirmed by our results showing that overexpression of \textit{SIDREB} in tomato leads to lower endogenous GA levels and consequently a dwarf phenotype, which enhances drought tolerance, and it is strengthened by the findings of (Magome et al., 2004), who also observed increased survival of GA-deficient \textit{ga1-3} mutant \textit{Arabidopsis} plants exposed to salt stress and who showed that the mutants exhibited a reduced growth phenotype, which was inhibited by a reduction in GA synthesis. Thus, it can be concluded that plants need adequate GA to promote developmental changes, but limiting GA production is also necessary for them to survive under unfavourable environments. Further studies on \textit{SIDREB} can provide a better understanding of how genes integrate environmental signals to restrict plant internode elongation by balancing ethylene, GA, and ABA signals.

\textbf{Supplementary data}

Supplementary data are available at \textit{JXB} online.

Supplementary Fig. S1. \textit{SpDREB} expression in response to drought, salt, and cold stress factors.

Supplementary Fig. S2. Multiple alignments of the predicted protein sequence of \textit{SIDREB} and DREBs from other plants.

Supplementary Fig. S3. Sequence of the promoter region of \textit{SICPS}.

Supplementary Table S1. Primer sequences used for real-time RT-PCR analysis of \textit{SIDREB} and genes from the GA biosynthesis and GA response pathways in tomato.

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