RESEARCH PAPER

The AP2/ERF transcription factor SlERF52 functions in flower pedicel abscission in tomato

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Abstract

In plants, abscission removes senescent, injured, infected, or dispensable organs. Induced by auxin depletion and an ethylene burst, abscission requires pronounced changes in gene expression, including genes for cell separation enzymes and regulators of signal transduction and transcription. However, the understanding of the molecular basis of this regulation remains incomplete. To examine gene regulation in abscission, this study examined an ERF family transcription factor, tomato (Solanum lycopersicum) ETHYLENE-RESPONSIVE FACTOR 52 (SlERF52). SlERF52 is specifically expressed in pedicel abscission zones (AZs) and SlERF52 expression is suppressed in plants with impaired function of MACROCALYX and JOINTLESS, which regulate pedicel AZ development. RNA interference was used to knock down SlERF52 expression to show that SlERF52 functions in flower pedicel abscission. When treated with an abscission-inducing stimulus, the SlERF52-suppressed plants showed a significant delay in flower abscission compared with wild type. They also showed reduced upregulation of the genes for the abscission-associated enzymes cellulase and polygalacturonase. SlERF52 suppression also affected gene expression before the abscission stimulus, inhibiting the expression of pedicel AZ-specific transcription factor genes, such as the tomato WUSCHEL homologue, GOBLET, and Lateral suppressor, which may regulate meristematic activities in pedicel AZs. These results suggest that SlERF52 plays a pivotal role in transcriptional regulation in pedicel AZs at both pre-abscission and abscission stages.

Key words: Abscission, abscission zone, cell-wall hydrolytic enzyme, ERF, functional switching, meristem, tomato, transcription activator, transcription factor.

Introduction

In plants, organ abscission specifically detaches senescent, injured, infected, or dispensable leaves or flower organs to maintain the healthy growth of the main body. Abscission also detaches mature seeds or fruits to disperse the plant’s progeny. To abscise an organ, plants generally develop a specialized tissue, the abscission zone (AZ), at a predetermined site on the organ to be abscised. Under normal conditions, the AZ firmly attaches the organ to the plant body; after initiation of abscission, the AZ tissues weaken, allowing the organ to detach. Plant hormones act in opposition to regulate organ separation: ethylene promotes abscission and auxin inhibits abscission, in an ethylene-antagonistic manner (Taylor and Whitelaw, 2001; Meir et al., 2010). Abscission involves the activation of cell-wall-degradation machinery in the AZ, including cell-wall hydrolytic enzymes such as endo-β-1,4-glucanase (also referred as cellulase (Cel)), polygalacturonase (PG), expansin, and xyloglucan endotransglucosylase/hydrolase (Roberts et al., 2002; Nakano and Ito, 2013). These enzymes degrade the primary cell wall or middle lamella pectin of AZ tissues so that abscising organs detach easily from the parent plant. Marked changes in transcription activate cell-wall degradation and other abscission processes (Meir et al., 2010; Wang et al., 2013); therefore, unveiling the mechanisms of transcriptional regulation will enable a more
clear understanding of the onset of abscission. In Arabidopsis thaliana, various transcription factors (TFs) positively or negatively regulate abscission of floral organs, including stamens, petals, and sepals. These TFs include members of the KNOTTED-LIKE HOMEBOX (KNOX) family, the DNA BINDING WITH ONE FINGER (DOF) family, the MADS-box family, the ETHYLENE-RESPONSIVE FACTOR (ERF) family, the AUXIN RESPONSE FACTOR (ARF) family, and the ZINC FINGER family (Fernandez et al., 2000; Ellis et al., 2005; Cai and Lashbrook, 2008; Wei et al., 2010; Chen et al., 2011; Shi et al., 2011a,b). However, the relationships among these TFs and the resulting transcriptional cascades remain incompletely understood.

Tomato (Solanum lycopersicum) plants develop AZs at the midpoint of the flower pedicels. The AZs have a knuckle-like structure with a groove on the surface. If pollination fails, the flower will senesce and eventually abscise from the plant at the AZ. During flower pedicel abscission, expression of PG and Cel greatly increases (Meir et al., 2010; Nakano et al., 2013; Wang et al., 2013). Programmed cell death also occurs during flower pedicel abscission (Bar-Dror et al., 2011). In tomato, several mutations can inhibit development of pedicel AZs, causing a ‘jointless’ phenotype. For example, jointless (j) is a mutation of a MADS-box TF gene and lateral suppressor (ls) is a mutation of a GRAS family TF gene (Schumacher et al., 1999; Mao et al., 2000). The locus for another ‘jointless’ mutation, j-2, has not yet been identified, but a sequencing analysis has identified a candidate gene encoding C-terminal domain (CTD) phosphatase-like 1 (ToCPL1) (Yang et al., 2005). In addition, the current study group has shown that the MADS-box TF MACROCALYX (MC) regulates pedicel AZ development and that a heterodimer of MC and J functions as a unit for this regulation (Nakano et al., 2012). Recent work identified another tomato MADS-box TF gene, SIMBP21, as a regulator of pedicel AZ development and showed that the encoded protein also interacts with MC and J (Liu et al., 2014). To identify more genes involved in pedicel abscission, Nakano et al. (2012, 2013) identified genes that are regulated by both MC and J and are expressed specifically in pedicel AZs. Interestingly, the results of this screen suggested that the tomato WUSCHEL homologue (LeWUS), GOBLET (GOB), Ls, and BLIND (Bl), which regulate meristem activity, also regulate pedicel AZ activity. However, their detailed roles in AZs remain unknown. The screen also identified several other TF genes: OIVATE, SIERFS52, and a zinc-finger-homeodomain (ZF-HD) family protein.

Based on the previous study, the current work focused on an ERF family TF gene, SIERFS52. The ERF family TFs constitute one of the largest TF families in the plant kingdom (Riechmann et al., 2000); for example, the tomato genome includes at least 85 genes for ERF family proteins, most of which remain uncharacterized (Sharma et al., 2010). The ERF family members contain a single DNA-binding domain, the APETALAL2 (AP2)/ERF domain (Ohme-Takagi and Shinshi, 1995), and, as monomers, recognize the GCC-box or CRT/DRE (for C-repeat/dehydration responsive element) cis-acting DNA elements (Allen et al., 1998; Hao et al., 1998; Yang et al., 2009). The AP2/ERF domain was identified in proteins binding to ethylene-responsive gene promoters (Ohme-Takagi and Shinshi, 1995), but subsequent studies revealed that the ERF family TFs function in diverse aspects of plant growth, development, and physiology, such as meristem activity, floral organ abscission, lipid metabolism, alkaloid biosynthesis, and responses to environmental stress (extreme temperature, water deficit, salinity, low oxygen, and pathogen infection) (Stockinger et al., 1997; Liu et al., 1998; Solano et al., 1998; van der Fits and Memelink, 2000; Banno et al., 2001; Berrocal-Lobo et al., 2002; Gu et al., 2002; Kirch et al., 2003; Komatsu et al., 2003; Broun et al., 2004; Xu et al., 2006; Shoji et al., 2010; Iwase et al., 2011). The current study used gene suppression to investigate the function of SIERFS52. The results demonstrate that SIERFS52 is required for activation of cell-wall-degrading enzymes during abscission as well as pedicel-specific gene expression at the pre-abscission stage.

Materials and methods

Plant materials

The tomato cultivar Ailsa Craig was used to make transgenic plants. The jointless mutant (TK3043) and the MC-suppressed transgenic plants were described previously (Nakano et al., 2012). Plants were grown in a controlled growth room under a 16/8 light/dark cycle at 25°C.

Plasmid construction

Oligonucleotide primers used for gene amplification are listed in Supplementary Table S1 (available at JXB online). To obtain the SIERFS52 gene fragments, cDNAs were synthesized from flower pedicel total RNA and used as templates for PCR amplification. A plasmid for RNA interference (RNAi) targeting SIERFS52 was constructed as follows. A 315-bp fragment of SIERFS52 was amplified with a pair of gene-specific primers, AK327476-F2 and AK327476-R2, and then cloned into the pENTR/D-TOPO Gateway entry vector (Invitrogen). The cloned fragment was transferred into a binary vector for RNAi, pBl-sense, anti-sense-GW (Inplanta Innovations, Japan) using Gateway LR Clonase Enzyme Mix (Invitrogen). The resultant plasmid was designated pBI-GW-SIERFS52-RNAi.

Plasmids for the transactivation assay were constructed as follows. The full-length open reading frame of SIERFS52 was amplified with the primer pair NcoI-SIERFS52-F1 and BamHI-SIERFS52-R1 and inserted into NcoI and BamHI sites of pGBK7 (Clontech), which carries an auxotrophic marker gene (TRP1). The resulting plasmid was designated pGBK-SIERFS52. Sequencing analysis revealed that SIERFS52 from Ailsa Craig possesses five single-nucleotide polymorphisms in comparison with the genome sequence of the cultivar Heinz 1706 (accession no. AB889741). A series of partial SIERFS52 fragments were amplified using NcoI-SIERFS52-F1 and BamHI-SIERFS52-R2 for amino acids 1–74, NcoI-SIERFS52-F1 and BamHI-SIERFS52-R3 for amino acids 1–98, NcoI-SIERFS52-F1 and BamHI-SIERFS52-R4 for amino acids 1–133, and NdeI-SIERFS52-C3 and BamHI-SIERFS52-R1 for amino acids 133–162. Each amplified DNA fragment was inserted into pGBK7, resulting in pGBK7-SIERFS52-R1, pGBK7-SIERFS52-R2, and pGBK7-SIERFS52-R3, respectively.

Plant transformation

The plant transformation vector pBI-GW-SIERFS52-RNAi was introduced into Agrobacterium tumefaciens EHA105 by the freeze–thaw method (Cindy and Jeff, 1994). Cotyledons of tomato seedlings were used for transformation by Agrobacterium infection according to the previously described method (Sun et al., 2006).
Transactivation assay

Transactivation assays in yeast cells were conducted according to the previously described method (Cho et al., 1999). The yeast strain AH109 (Clontech), which carries two auxotrophic marker genes (ADE2 for adenine biosynthesis and HIS3 for histidine biosynthesis) under the GAL4 cis-regulatory element, was used for the experiment. Yeast transformation was performed using the Frozen EZ Yeast Transformation II kit (Zymo Research, Irvine, CA, USA), and transformants were selected on SD media lacking tryptophan (SD/–Trp), adenine, and histidine (SD/–Trp/–Adel–His). In the experiment, a target protein was expressed as a fusion protein with the GAL4 DNA-binding domain (GAL4BD). PCR amplifications for the sequences used in the analysis. Supplementary Table S2 shows the accession numbers for the sequences used in the analysis.

Reverse-transcription PCR and quantitative reverse-transcription PCR

Total RNAs were extracted using the RNeasy mini kit (Qiagen) in combination with the QIA shredder spin column (Qiagen). First-strand cDNA was synthesized using PrimeScript II 1st strand cDNA Synthesis Kit (Takara Bio, Japan). PCR amplifications were performed using the ExTaq polymerase (Takara Bio). qRT-PCR was carried out with a 7300 Real-Time PCR System (Applied Biosystems) using THUNDERBIRD SYBR qPCR MIX (Toyobo, Japan). Data were normalized to the expression of the SAND (Toyobo, Japan) was used for the analysis. Supplementary Table S2 shows the accession numbers for the sequences used in the analysis.

Flower pedicel abscission assay

Flower pedicels were harvested at anthesis. The flower was removed from the pedicel using a sharp blade, the pedicel end was inserted into a 1.0% agar plate, and the plate was placed in a glass chamber to maintain high humidity. An abscission event was defined by pedicel detachment that occurred naturally or in a response to vibration applied to the distal portion of the explant.

Results

SlERF52 is a member of the ERF transcription factor family

As described previously, SlERF52 expression is strictly limited to the AZ region in the pedicel and SlERF52 expression is suppressed in plants that lack an AZ, namely MC-knockdown plants and j mutants (Nakano et al., 2012, 2013; Fig. 1A and B). No or very low expression of SlERF52 was detected in other organs, including roots, leaves, stems, flowers, sepals, and fruits (Fig. 1C). These results suggest that SlERF52 plays a specific role in pedicel abscission.

Phylogenetic analysis of the AP2/ERF domain revealed that SlERF52 belongs to group Va of ERFs (Fig. 2A). This group includes: Arabidopsis WAX INDUCER 1 (WIN1)/SHINE1 (SHN1), SHN2, and SHN3, which regulate cutin biosynthesis and abscission of floral organs (Aharoni et al., 2004; Broun et al., 2004; Shi et al., 2011b); the tomato homologue of SHN3 (SISHN3) (Shi et al., 2013); barley (Hordeum vulgare) NUDUM (NUD), which regulates lipid biosynthesis for hull-caryopsis adhesion of grain (Taketa et al., 2008); tomato LeERF1, which regulates ethylene signalling (Li et al., 2007); Medicago truncatula ERF REQUIRED FOR NODULE DIFFERENTIATION (EFD) (Vernie et al., 2008); and popular (Populus tremula × P. alba) PtaERF003, which is involved in adventitious and lateral root formation (Trupiano et al., 2013). Group Va ERFs have three conserved domains: the AP2/ERF domain, conserved motif V (CMV)-1, and CMV-2 in adventitious and lateral root formation (Trupiano et al., 2013). Group Va ERFs have three conserved domains: the AP2/ERF domain, conserved motif V (CMV)-1, and CMV-2

Fig. 1. Expression specificity of SlERF52. (A) Expression analysis of SlERF52 in a jointless mutant, a MC-suppressed transgenic plant (AS-MC), and the wild type (WT). (B) Expression specificity of SlERF52 within flower pedicel parts, the distal (Dis), proximal (Prox), and abscission zone (AZ) regions in WT anthesis flowers. (C) Expression analysis of SlERF52 among various organs. Expression analysis was performed by reverse-transcription PCR using SAND (A and B) and SlActin-51 (C) as the internal control.

SlERF52 acts as a positive regulator of flower pedicel abscission

To analyse the biological role of SlERF52, RNAi was used to knock down SlERF52 expression. To that end, transgenic plants with an RNAi vector targeting SlERF52 were generated, 15 independent transgenic plants were obtained, and the three plants with the lowest expression levels of SlERF52 (plants 7, 18, and 20) were selected for further analysis (Fig. 3A and Supplementary Fig. S1). The three SlERF52-suppressed plants appeared similar to wild-type plants and developed pedicel AZs normally (Fig. 3B), indicating that SlERF52 does not regulate differentiation of pedicel AZs. To examine the pedicel abscission behaviour of the transgenic
plants, flower pedicel abscission was induced by removing the flower from the pedicel, which stimulates ethylene production and restricts auxin supply from the flower (Meir et al., 2010) and observing the frequency of abscission in the flower-removed pedicels for 3 d (Fig. 3C). The abscission frequency of pedicels from plants 7 and 20 at 3 d after flower removal was significantly lower than that of wild type, indicating that the pedicels of the two suppression lines showed decreased abscission potential compared to the wild type (Fig. 3D). The pedicels from plant 18 exhibited significant reduction of abscission frequency at 1 d after flower removal, although the abscission eventually occurred at the same level as the wild type at 3 d after flower removal (Fig. 3D). These observations indicate that the suppression of \textit{SlERF52} impaired activation of pedicel abscission.

**Suppression of \textit{SlERF52} inhibits induction of genes for cell-wall hydrolytic enzymes**

Expression of genes encoding cell-wall hydrolytic enzymes, including PG and Cel, is induced in response to the abscission stimulus (Roberts et al., 2002). Because suppression of \textit{SlERF52} decreased the rate of pedicel abscission, the current work investigated whether it also affected the transcript levels of genes encoding PG (\textit{TAPG1}, \textit{TAPG2}, and \textit{TAPG4}) and Cel (\textit{Cell} and \textit{Cel5}) during flower pedicel abscission. In accord with previous reports (Meir et al., 2010; Nakano et al., 2013; Wang et al., 2013), in wild-type plants, removal of the flower induced the expression of \textit{TAPG1}, \textit{TAPG2}, \textit{TAPG4}, \textit{Cell}, and \textit{Cel5} in AZs, but \textit{SlERF52} was expressed at constant levels before and after the onset of abscission (Fig. 4). In \textit{SlERF52}-suppressed plants 7 and 20, \textit{TAPG1}, \textit{TAPG2}, \textit{TAPG4}, and \textit{Cel5} were induced to significantly lower levels than in the wild type (Fig. 4), and the levels of these four genes corresponded to the abscission rates in the suppressed transformants (Fig. 3). The suppression was more severe for \textit{PG} genes than for \textit{Cel5}. Meanwhile, the levels of \textit{Cell} expression did not correspond to the abscission rate.

**Suppression of \textit{SlERF52} reduces expression of transcription factor genes \textit{LeWUS}, \textit{GOB}, and \textit{Ls} in pedicel AZs**

Previously, this study group reported that \textit{LeWUS}, \textit{GOB}, \textit{Ls}, and \textit{Bl}, four TF genes associated with shoot apical meristem or axillary meristem function, might also be involved in the regulation of pedicel AZ activity (Nakano et al., 2012, 2013). To investigate whether \textit{SlERF52} affects the expression of these four TF genes, their transcript levels in the \textit{SlERF52}-suppressed plants were analysed. As observed previously, in wild-type plants, the expression of \textit{LeWUS}, \textit{GOB}, and \textit{Ls} decreased markedly in response to flower removal,
SlERF52 functions in flower pedicel abscission. In the SlERF52-suppressed plants, however, the transcript levels of these three genes were much lower than the wild type before flower removal (0 d) and their levels remained low after flower removal (1 d and 2 d) (Fig. 4). By contrast, the expression of Bl increased during abscission similarly in the SlERF52-suppressed plants and the wild type (Fig. 4). The transcript level of Bl in the suppressed plants was slightly lower than that in wild type throughout the examined period but the difference was not significant, except in the d-1 samples. The expression pattern of these four TF genes was not correlated with the expression of SlERF52 in shoot apices or leaf axillae of wild type plants and also was not affected by suppression of SlERF52 (Supplementary Fig. S2), which is consistent with the normal vegetative growth of the suppressed plants. The results suggest that the SlERF52-mediated regulation of LeWUS, GOB, and Ls is specific to pedicel AZs.

### Discussion

**SlERF52 functions as a transcriptional activator**

ERF proteins can activate or repress transcription of target genes (Fujimoto et al., 2000; Ohta et al., 2001). This study investigated the transcriptional activation potential of SlERF52 using a yeast system, with the GAL4 DNA-binding domain (DBD) fused to SlERF52 and marker genes expressed under the control of the GAL4 target-binding site. The results showed that the construct with the full-length SlERF52 coding region (GAL4DBD-SIERF52_1–162) induced expression of the marker genes (Fig. 5), indicating that SlERF52 can activate transcription. To identify which region of SlERF52 is necessary for the activity, three truncated SlERF52 proteins (SlERF52_1–74, SlERF52_1–98, and SlERF52_1–133) were assayed, but no activity was detected in any of the C-terminal truncated proteins (Fig. 5). By contrast, this work did detect activity in a construct with the C-terminal 30 amino acids (GAL4DBD-SIERF52_133–162) (Fig. 5). These results indicated that the transcriptional activation activity of SlERF52 requires the C-terminal 30-amino-acid region that contains the CMV-2 motif.

These data showed that suppression of SlERF52 reduced the rate of pedicel abscission and repressed induction of the genes for cell-wall hydrolytic enzymes PG and Cel (Cel5, TAPG1, TAPG2, and TAPG4). Abscission of flower pedicels and leaf petioles in tomato requires the activity of these enzymes...
These results also indicate that the transcript level of *Cel1* or the abscission rate related with suppression of *Cel1* in the suppressed plants, the expression of *Cel1* was not correlated with suppression of *SIERF52* or the abscission rate. These results also indicate that the transcript level of *Cel1* in the wild type peaked at 1 d after flower removal and then declined, but the transcript levels of *Cel5*, *TAPG1*, *TAPG2*, and *TAGP4* continuously increased (Fig. 4). In addition, *Cel1* is expressed in a pedicel region distinct from the region where *TAPG1* and *TAPG4* are expressed (Bar-Dror et al., 2011). These results imply that the transcriptional regulation of *Cel1* is independent of the regulation mediated by *SIERF52*. Therefore, these results indicate that *SIERF52* acts as a key positive regulator of flower pedicel abscission, but abscission also involves a *SIERF52*-independent pathway.

Interestingly, *SIERF52* is necessary, but not sufficient, for the upregulation of *PG* and *Cel* genes; before the onset of abscission, *SIERF52* is also expressed at a similar level to that observed after flower removal, but this expression does not induce *PG* and *Cel* gene expression (Fig. 4). Post-transcriptional regulation may explain the transcription-independent activity of *SIERF52* (as will be discussed).

**Fig. 4.** Expression analysis in *SIERF52*-RNAi plants during abscission. Pedicel abscission was induced by anthesis flower removal and gene expression was investigated for 2 d by quantitative reverse-transcription PCR. For single RNA sample preparation, 3–24 pedicel abscission zones, which include both attached and abscised pedicels, were harvested in bulk and used for the analysis. Levels of transcripts are shown as fold-change values relative to the 0 d sample of WT (for *Cel1*, *Cel5*, *TAPG4*, *SIERF52*, *Bl*, *GOB*, *LeWUS*, and *Ls*). Because *TAPG1* and *TAPG2* transcript levels for the 0 d sample of WT were below detection limit (shown as ND), the level of the two genes are shown relative to the sample of *SIERF52*-suppressed plant 20 at 1 d. Data are means±SD of biological triplicates.

(Slashbrook et al., 1998; Jiang et al., 2008). Therefore, these results suggest that *SIERF52* induces pedicel abscission through upregulation of these enzyme genes. In contrast to the low induction of *Cel5*, *TAPG1*, *TAPG2*, and *TAGP4* in the suppressed plants, the expression of *Cell* was not correlated with suppression of *SIERF52* or the abscission rate. SIERF52 is involved in the expression of TF genes for shoot apical meristem and axillary meristem function in flower pedicel AZs

LeWUS, GOB, Ls, and Bl, key TF genes for meristem-associated functions, are expressed specifically in flower pedicel AZs, suggesting that these four TFs may have an additional function in control of organ abscission through regulation.
of meristem-like activity in the cells within the AZ (Nakano et al., 2012, 2013). The current study found that LeWUS, GOB, and Ls were expressed at significantly lower levels in the SlERF52-suppressed plants, implying that SlERF52 may be involved in the regulation of these TF genes. Expression of SlERF52, LeWUS, GOB, and Ls is reduced in pedicels of MC-suppressed plants, SIMBP21-suppressed plants, and f mutants (Nakano et al., 2012; Liu et al., 2014; Fig. 1A), indicating that SlERF52 may mediate the effect of MC, J, and SIMBP21 on these meristem-associated regulators. Two SlERF52 homologues that belong to the incomplete CMV-1 type subgroup regulate plant development through modulation of meristem activity: medicago EFD controls formation of root nodule meristems (Vernie et al., 2008) and poplar PtaERF003 controls formation and growth of adventitious and lateral root meristems (Trupiano et al., 2013). Therefore, the control of meristem-associated regulation may be a conserved biological function for the group Va ERFs with incomplete CMV-1 motifs. PtaERF003 functions in an auxin-regulated pathway that regulates root meristems (Trupiano et al., 2013). Similar to root meristem regulation, expression of the shoot meristem-associated TF genes in the AZs may be regulated by a signalling pathway that requires auxin supplied from the flower before the onset of abscission, and SlERF52 may function in the auxin signalling pathway in the AZs.

The expression analyses revealed that SlERF52 activates the expression of LeWUS, GOB, and Ls in the AZ cells, but the expression of these three TF genes was suppressed after stimulation of abscission, even though SlERF52 expression remained constant (Fig. 4). By contrast, the cell-wall hydrolytic enzyme genes were suppressed before the stimulation of abscission, even though SlERF52 expression remained constant, a reverse pattern to that of the three TF genes. This partial dependence on SlERF52 is discussed in the next section.

Of the four TF genes for meristem-associated functions, BII exhibits significant upregulation after flower removal, an expression pattern distinct from LeWUS, GOB, and Ls (Fig. 4). Thus, Nakano et al. (2013) hypothesized that an independent pathway controls Bl expression, although MC and J are involved in the expression of all four TF genes. In the current study, the suppression of SlERF52 did not significantly affect Bl expression, indicating that a SlERF52-independent pathway regulates Bl. Also, the intense induction of Bl after flower removal suggests that Bl may be involved in pedicel abscission (Nakano et al., 2013). The induction of Bl in the SlERF52-suppressed plants may help explain the partial progression of abscission in the suppressed lines.

Functional switching of SlERF52 before and after the onset of abscission

SlERF52 functions in the regulation of pedicel abscission and regulates transcription of distinct sets of genes before and after the onset of abscission. In the pre-abscission stage, the expression of LeWUS, GOB, and Ls requires SlERF52, either directly or indirectly. In response to an abscission-inducing stimulus, the expression of Cel5, TAPG1, TAPG2, and TAPG4 was also regulated by SlERF52, directly or indirectly. However, after the onset of abscission, the induction of LeWUS, GOB, and Ls ceases. To explain how SlERF52 is involved in the regulation of distinct sets of genes before and after the onset of abscission, it is postulates that coregulators specify the function of SlERF52 in the different states. In this hypothesis, SlERF52 recruits state-specific TFs and each state-specific TF complex activates expression of a distinct set of target genes. Several ERFs are predicted to require cofactors to bind target genes (Chakrarthy et al., 2003; Kannangara et al., 2007; Cheng et al., 2013). As another possibility, repressor proteins or chromatin remodelling at SlERF52-binding sites may restrict the transactivation activity of SlERF52 in a stage-specific manner.

This work used knockdown experiments to examine SlERF52 function. A recent study using overexpression of SIMBP21 provided substantial insights on SIMBP21 gene function, adding to the results of the knockdown assay (Liu et al., 2014). However, unlike the study of SIMBP21,
overexpression of SIERF52 may not be effective to clarify SIERF52 function because the activity of SIERF52 in AZs is likely determined by other factors associated with SIERF52, not by the expression level of SIERF52.

In conclusion, the results of this study demonstrated that SIERF52 regulates pedicel AZ-specific transcription at both pre-abscission and abscission stages and that the regulation during the latter stage includes some of the genes required for abscission. The functional switching between before and after the onset of abscission, by a still-unknown mechanism, raises the possibility that SIERF52 serves as a hub TF that regulates the phase transition between the two stages. The identification of the switching mechanism will further improve the understanding of abscission.

Supplementary material

Supplementary data are available at *JXB* online.

**Supplementary Table S1.** Sequences of the oligonucleotide primers used in this study.

**Supplementary Table S2.** Accession numbers of ERFs used for construction of the phylogenetic tree.

**Supplementary Fig. S1.** Expression analysis of SIERF52-RNAi transgenic plants.

**Supplementary Fig. S2.** Expression of SIERF52 and meristem-associated TF genes in shoot apex and leaf axilla.

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