The AP2/ERF transcription factor SIERF52 functions in flower pedicel abscission in tomato

Toshitsugu Nakano, Masaki Fujisawa, Yoko Shima and Yasuhiro Ito*

National Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305–8642, Japan

* To whom correspondence should be addressed. E-mail: yasuito@affrc.go.jp

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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 (SIERF52). SIERF52 is specifically expressed in pedicel abscission zones (AZs) and SIERF52 expression is suppressed in plants with impaired function of MACROCALYX and JOINTLESS, which regulate pedicel AZ development. RNA interference was used to knock down SIERF52 expression to show that SIERF52 functions in flower pedicel abscission. When treated with an abscission-inducing stimulus, the SIERF52-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. SIERF52 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 SIERF52 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 endotransglycosylase/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 sepalas. These TFs include members of the KNOTTED-LIKE HOMEobox (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 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 showed 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: OVAE, SIERF52, and a zinc finger-homeodomain (ZF-HD) family protein.

Based on the previous study, the current work focused on an ERF family TF gene, SIERF52. 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 APETALA2 (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 SIERF52. The results demonstrate that SIERF52 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 SIERF52 gene fragments, cDNAs were synthesized from flower pedicel total RNA and used as templates for PCR amplification. A plasmid for RNA interference (RNAi) targeting SIERF52 was constructed as follows. A 315-bp fragment of SIERF52 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, pBi-sense, anti sense-GW (Inplanta Innovations, Japan) using Gateway LR Clonase Enzyme Mix (Invitrogen). The resultant plasmid was designated pBI-GW-SIERF52-RNai.

Plasmids for the transactivation assay were constructed as follows. The full-length open reading frame of SIERF52 was amplified with the primer pair NcoI-SIERF52-F1 and BamHI-SIERF52-R1 and inserted into the NcoI and BamHI sites of pGBK7 (Clontech), which carries an auxotrophic marker gene (TRPI). The resulting plasmid was designated pGBK-SIERF52. Sequencing analysis revealed that SIERF52 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 SIERF52 fragments were amplified using NcoI-SIERF52-F1 and BamHI-SIERF52-R2 for amino acids 1–74, NcoI-SIERF52-F1 and BamHI-SIERF52-R3 for amino acids 1–98, NcoI-SIERF52-F1 and BamHI-SIERF52-R4 for amino acids 1–133, and NdeI-SIERF52-C3 and BamHI-SIERF52-R1 for amino acids 133–162. Each amplified DNA fragment was inserted into pGBK7, resulting in pGBK7-SIERF52, pGBK7-SIERF52, pGBK7-SIERF52, and pGBK7-SIERF52, respectively.

Plant transformation

The plant transformation vector pBI-GW-SIERF52-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 medium lacking tryptophan (SD−Trp, Clontech). Assays for transactivation activity were performed on SD medium lacking tryptophan, 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), and if the protein had the potential to activate transcription, the auxotrophic marker genes (ADE2 and HIS3) were expressed and the yeast cell was able to grow on the adenine- and histidine-deficient selection medium.

Sequence analysis

Multiple sequence alignment was performed with ClustalW version 1.83 and the phylogenetic tree was constructed by the neighbour-joining method. GENETYX version 10 (GENETYX, Japan) was used for 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 RNasy 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 gene (SGN-U316474) as an internal control (Exposito-Rodriguez et al., 2008). Relative quantification of expression of each gene was performed using the 2^−ΔΔCT method (Livak and Schmittgen, 2001).

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

**SIERF52 is a member of the ERF transcription factor family**

As described previously, SIERF52 expression is strictly limited to the AZ region in the pedicel and SIERF52 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 SIERF52 was detected in other organs, including roots, leaves, stems, flowers, sepals, and fruits (Fig. 1C). These results suggest that SIERF52 plays a specific role in pedicel abscission.

Phylogenetic analysis of the AP2/ERF domain revealed that SIERF52 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., 2011); 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 at the C-terminus (Fig. 2B). Group Va includes two subgroups, a subgroup with the normal CMV-1 domain (including LeERF1, the AT5G15190-encoding protein, EFD, PtaERF003, and 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 at the C-terminus (Fig. 2B). Group Va includes two subgroups, a subgroup with the normal CMV-1 domain (including LeERF1, the AT5G15190-encoding protein, EFD, PtaERF003, and SIERF52; Fig. 2B).

**SIERF52 acts as a positive regulator of flower pedicel abscission**

To analyse the biological role of SIERF52, RNAi was used to knock down SIERF52 expression. To that end, transgenic plants with an RNAi vector targeting SIERF52 were generated, 15 independent transgenic plants were obtained, and the three plants with the lowest expression levels of SIERF52 (plants 7, 18, and 20) were selected for further analysis (Fig. 3A and Supplementary Fig. S1). The three SIERF52-suppressed plants appeared similar to wild-type plants and developed pedicel AZs normally (Fig. 3B), indicating that SIERF52 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 \( \text{SlERF52} \) impaired activation of pedicel abscission.

**Suppression of SIERF52 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 \( \text{SIERF52} \) decreased the rate of pedicel abscission, the current work investigated whether it also affected the transcript levels of genes encoding PG (\( \text{TAPG1, TAPG2, and TAPG4} \)) and Cel (\( \text{Cell} \) and \( \text{Cel5} \)) 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 \( \text{TAPG1, TAPG2, TAPG4, Cell1, and Cel5} \) in AZs, but \( \text{SIERF52} \) was expressed at constant levels before and after the onset of abscission (Fig. 4). In \( \text{SIERF52} \)-suppressed plants 7 and 20, \( \text{TAPG1, TAPG2, TAPG4, and Cel5} \) 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 PG genes than for \( \text{Cel5} \). Meanwhile, the levels of \( \text{Cell} \) expression did not correspond to the abscission rate.

**Suppression of SIERF52 reduces expression of transcription factor genes LeWUS, GOB, and Ls in pedicel AZs**

Previously, this study group reported that \( \text{LeWUS, GOB, Ls, and Bl} \), 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 \( \text{SIERF52} \) affects the expression of these four TF genes, their transcript levels in the \( \text{SIERF52} \)-suppressed plants were analysed. As observed previously, in wild-type plants, the expression of \( \text{LeWUS, GOB, and Ls} \) decreased markedly in response to flower removal,
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an abscission stimulus. 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.

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-SlERF521–162) 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 (SlERF521–74, SlERF521–98, and SlERF521–133) 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-SlERF52133–162) (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.

Discussion

SlERF52 functions as a positive regulator of flower pedicel abscission

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 \textit{SlERF52} or the abscission rate, related with suppression of \textit{Cel1}, was not correlated with that observed after flower removal, but this expression does not induce \textit{PG} and \textit{Cel} gene expression (Fig. 4). Post-transcriptional regulation may explain the transcription-independent activity of \textit{SlERF52} (as will be discussed).

\textit{SlERF52}, a positive regulator of abscission, has an opposite role to that of the \textit{Arabidopsis} homologues, \textit{SHNs}, which act as negative regulators of abscission of floral organs such as sepals, stamens, and petals (Shi et al., 2011b). Simultaneous suppression of all \textit{SHN} genes induces earlier abscission of floral organs, possibly due to decreased cutin deposition and altered cell-wall composition of structural proteins and pectin (Shi et al., 2011b). \textit{SlERF52} and \textit{SHNs} belong to the group Va \textit{ERF} family, but belong to different subgroups based on their CMV-1 motif structures: \textit{SlERF52} belongs to the subgroup with an incomplete CMV-1, and the \textit{SHNs} belong to the subgroup with normal CMV-1 structure (Fig. 2B). The biological function of CMV-1 has not been identified, but the structural difference in CMV-1 between \textit{SlERF52} and \textit{SHNs} may be a possible cause of their functional diversity. Also, the latter half of the CMV-1 motif, which is lost in the incomplete-type Va \textit{ERF}s, contains an important active site, as demonstrated in a study of mutants of \textit{NUD}, a barley orthologue of \textit{WIN1/SHN1} (Fig. 2B; Taketa et al., 2008). Elucidation of the function of the CMV-1 motif will provide insights into the functional diversity between the subgroups within the Va \textit{ERFs}, including \textit{SlERF52} and \textit{SHNs}.

Several group Va \textit{ERF} proteins, including \textit{WIN1/SHN1}, \textit{SHN2}, \textit{SHN3}, and \textit{EFD}, act as transcriptional activators (Vernie et al., 2008; Shi et al., 2011b). However, the domain for transcriptional activation was not identified. The current study demonstrated that the C-terminus of \textit{SlERF52}, which contains the CMV-2 motif, acts as an activation domain. As shown in Fig. 2B, the CMV-2 motif is highly conserved in \textit{WIN1/SHN1}, \textit{SHN2}, \textit{SHN3}, and \textit{EFD}, suggesting that the conserved motif functions as a transcription activation domain in these proteins.

\textit{SlERF52} is involved in the expression of TF genes for shoot apical meristem and axillary meristem function in flower pedicel AZs

\textit{LeWUS}, \textit{GOB}, \textit{Ls}, and \textit{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 SIERF52-suppressed plants, implying that SIERF52 may be involved in the regulation of these TF genes. Expression of SIERF52, LeWUS, GOB, and Ls is reduced in pedicels of MCV-suppressed plants, SIMBP21-suppressed plants, and j mutants (Nakano et al., 2012; Liu et al., 2014; Fig. 1A), indicating that SIERF52 may mediate the effect of MC, J, and SIMBP21 on these meristem-associated regulators. Two SIERF52 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 SIERF52 may function in the auxin signalling pathway in the AZs.

The expression analyses revealed that SIERF52 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 SIERF52 expression remained constant (Fig. 4). By contrast, the cell-wall hydrolytic enzyme genes were suppressed before the stimulation of abscission, even though SIERF52 expression remained constant, a reverse pattern to that of the three TF genes. This partial dependence on SIERF52 is discussed in the next section.

Of the four TF genes for meristem-associated functions, B1 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 B1 expression, although MC and J are involved in the expression of all four TF genes. In the current study, the suppression of SIERF52 did not significantly affect B1 expression, indicating that a SIERF52-independent pathway regulates B1. Also, the intense induction of B1 after flower removal suggests that B1 may be involved in pedicel abscission (Nakano et al., 2013). The induction of B1 in the SIERF52-suppressed plants may help explain the partial progression of abscission in the suppressed lines.

**Functional switching of SIERF52 before and after the onset of abscission**

SIERF52 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 SIERF52, either directly or indirectly. In response to an abscission-inducing stimulus, the expression of Cell5, TAPG1, TAPG2, and TAPG4 was also regulated by SIERF52, directly or indirectly. However, after the onset of abscission, the induction of LeWUS, GOB, and Ls ceases. To explain how SIERF52 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 SIERF52 in the different states. In this hypothesis, SIERF52 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 (Chakravarthy et al., 2003; Kannangara et al., 2007; Cheng et al., 2013). As another possibility, repressor proteins or chromatin remodelling at SIERF52-binding sites may restrict the transactivation activity of SIERF52 in a stage-specific manner.

This work used knockdown experiments to examine SIERF52 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 \textit{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 \textit{SIERF52}.

In conclusion, the results of this study demonstrated that \textit{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.

\section*{Supplementary material}

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

\textbf{Supplementary Table S1.} Sequences of the oligonucleotide primers used in this study.

\textbf{Supplementary Table S2.} Accession numbers of ERFs used for construction of the phylogenetic tree.

\textbf{Supplementary Fig. S1.} Expression analysis of \textit{SIERF52-RNAi} transgenic plants.

\textbf{Supplementary Fig. S2.} Expression of \textit{SIERF52} and meristem-associated TF genes in shoot apex and leaf axilla.

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