

Role of the KNOTTED1-LIKE HOMEBOX Protein (KD1) in regulating tomato flower pedicel abscission at early and late stages of the process

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Abstract

Background The *KNOTTED1-LIKE HOMEODOMAIN PROTEIN1* (*KD1*) gene is highly expressed in flower and leaf abscission zones (AZs). RNA-antisense silencing of *KD1* was shown to delay tomato pedicel and petiole abscission, induced by flower or leaf removal, respectively. *KD1* was found to regulate flower pedicel abscission via alteration of auxin gradient through the flower AZ (FAZ), and disruption of the auxin response at the early stages of the abscission process. The present work was aimed to further understand how *KD1* regulates signaling factors and regulatory genes involved in the delay of pedicel abscission using the silenced *KD1* lines. For this purpose we performed a large scale transcriptome profiling of the FAZ at various time points after flower removal, using a customized AZ-specific microarray.

Results The results highlighted a differential expression of regulatory genes in the FAZ of *KD1* -silenced plants compared to the wild type (WT). These genes were controlled by *KD1* before and after abscission induction. In the *KD1* -silenced plants, *KD1* expression already decreased at zero time before flower removal, resulting in altered expression of regulatory genes, including epigenetic modifiers, transcription factors (TFs), post-translation regulators, and antioxidative defense components. The increased expression of these regulatory genes, and genes related to exocytosis and gibberellin perception, observed in the WT FAZ, was inhibited in the *KD1*- silenced plants at 4 h after flower removal. This response led to an inhibited abscission phenotype and downregulation of genes involved in abscission execution and defense in the *KD1* -silenced plants.

Conclusions The data suggest that *KD1* is a master regulator in tomato flower abscission. Some of the altered genes might directly affect auxin homeostasis and transport, resulting in slowing down auxin depletion in the FAZ. This probably delayed the subsequent cascade of the molecular events described above. The data obtained suggest that the inhibitory effect of *KD1* silencing on flower pedicel abscission is not limited to manipulation of auxin levels and response as previously reported, but it probably also operates via alteration of other regulatory pathways that delay the acquisition of the competence of the FAZ cells to respond to ethylene signaling.

Background

It is well documented that removal of the leaf blades or flowers, the major sources of auxin production, or application of polar auxin transport inhibitors induce petiole or pedicel abscission, respectively, as a result of reduction in auxin transport to the organ abscission zone (AZ). This leads to altered expression of auxin-related genes and upregulation of ethylene-related genes [1] as well as to differential expression of other regulatory genes in the flower AZ (FAZ) of tomato (*Solanum lycopersicum*).. These events resulted in the acquisition of the FAZ cell competence to respond to ethylene signaling [1–5].

In a previous study, focused on the tomato pedicel abscission process induced by flower removal, we identified by transcriptome microarray analysis, several genes, the expression of which was modified in

the FAZ but remained unchanged in the pedicel non-AZ (NAZ) [2]. These genes were suggested to be involved in the acquisition of the FAZ cell competence to respond to ethylene signaling following auxin depletion [2]. Out of these FAZ-specifically expressed genes, two genes were further studied for their functional roles in abscission. These included the *KD1* gene, which encodes the KNOTTED1-LIKE HOMEODOMAIN PROTEIN1 that belongs to class I KNOX family [6], and the *THyPRP* gene, which encodes the Tomato Hybrid Proline-Rich Protein [7]. We previously showed that downregulation of *KD1* by antisense significantly delayed pedicel and petiole abscission in tomato plants by affecting auxin levels in the AZ and auxin gradient from the distal to the proximal region of the FAZ without impairing their growth and development [6]. Additionally, we recently reported that downregulation of *THyPRP* by silencing delayed pedicel abscission by regulating the FAZ cell competence to respond to ethylene signals [7]. The present study focused on further elucidation of the role of *KD1* in abscission, and understanding its mode of action, using new tools that were not available in our previous work.

Three knotted proteins, *KNAT1*, *KNAR2* and *KNAT6* in *Arabidopsis*, as well as the knotted *TKN4* in tomato, were previously demonstrated to be involved in flower organ abscission, acting as downstream factors of the INFLORESCENCE DEFICIENT IN ABSCISSION (*IDA*)-*HAESA* pathway [8–10]. Recently, a KNOX protein, *OSH15*, was demonstrated to enhance seed shattering in rice by directly inhibiting lignin biosynthesis genes [11]. Apart of being involved in abscission processes, *KD1* and *KNOX* genes have a well-documented role in leaf development, and are known to modulate boundary separation and proximal-distal axis development in leaves of dicots [12–14]. In monocots, the KNOX regulatory network was reported to play a role in shoot apical meristem maintenance in rice and maize [15, 16]. In different plant systems, the class I KNOX family proteins were found to regulate several genes involved in biosynthesis and signal transduction of various plant hormones, various TFs and lignin synthesis [17]. Recently, a KNOXI transcription factor (TF) was demonstrated to regulate floral fate in *Arabidopsis*, further clarifying the role of KNOX TFs in the specification of flower meristem identity [18].

Manipulating *KD1* expression affected various aspects of plant development, which were all related to the *KD1* role in auxin gradient responses [19], and the KNOX protein Knotted1 was reported to directly control the auxin signaling pathway in maize at all levels, including auxin synthesis, transport, and signaling [20]. Indeed, our previous work, which investigated the role of *KD1* in abscission, demonstrated a strong effect of *KD1* on the auxin gradient in the FAZ [6]. Thus, the *KD1* gene was silenced, under the control of the AZ-specific promoter, *Tomato Abscission Polygalacturonase4 (TAPG4)* [2, 6, 21, 22], to study the changes in gene expression profiles in the tomato FAZ shortly (4 h) after flower removal, in the *TAPG4::antisense KD1* plants compared to the wild type (WT). The data obtained showed that the involvement of *KD1* in abscission regulation was associated with auxin transporters and signaling components, and that the changes in *KD1* expression modulated the auxin concentration and response gradient in the FAZ. Reducing *KD1* transcript abundance, which is highly expressed in the AZs, delayed organ abscission, but it did not prevent the process, suggesting that *KD1* is part of a complex system controlling the abscission process [6].

Our previous study [6] raised some questions that remained unsolved. These included: a) how *KD1* is regulating the auxin-related genes; b) what is the role of *KD1* in regulation of other signaling factors and regulatory genes involved in the delay of pedicel abscission at zero time and at the early (4 h) and late (8–20 h) stages of the abscission process occurring after flower removal. To answer these questions, we performed in the present study a detailed transcriptome analysis of the FAZ and the NAZ of WT plants compared to the FAZ of the *TAPG4::antisense KD1* plants, at various time points after flower removal, using a newly developed customized AZ-specific microarray [22]. The use of this customized AZ-specific microarray in a time course analysis, allowed to expand the database of changes in gene expression occurring at the early stage of pedicel abscission, since it contains more gene probes than those that were previously used by Ma et al. [6]. The results obtained in the present study highlighted expression of regulatory genes in the FAZ, which seem to be controlled by *KD1* before and after abscission induction. These genes might have a role in regulating the acquisition of the FAZ cell competence to respond to ethylene signaling, or in controlling abscission in a different mode of action.

Results And Discussion

Downregulation of *KD1* in the AZ inhibits tomato pedicel and petiole abscission

Quantitative reverse transcription (qRT-PCR) demonstrated that the *KD1* transcript was predominately expressed in the FAZ, and its expression was downregulated to very low levels within 8 h after flower removal (Fig. 1). This rapid decrease in *KD1* expression in the tomato FAZ after flower removal was already demonstrated in our previous publications [2, 6]. Pretreatment with the ethylene inhibitor 1-methylcyclopropene (1-MCP) did not affect the expression of *KD1*, but application of IAA after flower removal prevented the decrease in its expression [2]. These data indicate that *KD1* expression in the FAZ is IAA-dependent, and that the decrease in its expression is a result of IAA depletion after flower removal. Similar results were reported for other Knotted family members, such as *Knotted TKN2/LET6* (AF000141) and *knotted TKN4* (AF533597) [2]. The involvement of three knotted proteins, KNAT1, KNAT2, and KNAT6, was also reported for the INFLORESCENCE DEFICIENT IN ABSCISSION (IDA)—HAE/HSL1-dependent flower organ abscission in Arabidopsis [8–10]. These observations suggest that *KD1* and *TKN4* could function as regulators of the acquisition of AZ cell competence to respond to ethylene signaling after IAA depletion.

The important role of *KD1* in tomato flower and leaf abscission, was previously demonstrated by virus-induced gene silencing (VIGS), and by antisense silencing under the AZ-specific promoter *TAPG4*, which led to delayed pedicel and petiole abscission [6]. The regulation of abscission by *KD1* was found to be associated with modulation of the auxin concentration and response in the FAZ, leading to changes in the abundance of genes related to auxin transporters and signaling components [6]. We used the same lines A and E of generation T4 in the present study.

The efficacy of *KD1* silencing is demonstrated by the qRT-PCR results (Fig. 2A). It is evident that the *TAPG4* promoter was very active in the FAZ [21], and *KD1* expression was downregulated by about 60% at zero time, and by about 70% at 4 h after flower removal, approximately at the same rates that were previously reported [6]. The microarray antisense probe showed increased expression of *KD1* between 4–20 h after flower removal (Fig. 2B). This antisense probe spanned the cloned fragment of *KD1* (Fig. 2C). We expected a higher expression of the antisense probe also at zero time (Fig. 2B), because the expression of *KD1* in the transgenic plants decreased by 60% at this time point (Fig. 2A). At present we do not have a reasonable explanation for this unexpected results.

The antisense transgenic lines exhibited a very significant delay in pedicel and petiole abscission following abscission induction (Fig. 3). All the pedicels in the WT plants abscised at 20 h after flower removal, compared to only 40% of abscised pedicels in the *TAPG4::antisense KD1* lines (Fig. 3A). Even at 48 h after flower removal, 25% of the pedicels in the transgenic plants still remained attached (data not shown). Petiole abscission of leaves at position 1 above the cotyledons reached 100% in the WT plants 4 days after leaf deblading and ethylene treatment, whereas in the *TAPG4::antisense KD1* plants it reached only 60 and 70% abscission rate in the silenced lines A and E, respectively (Fig. 3B). The inhibition of petiole abscission in the transgenic plants was more significant in leaf position 4 (Fig. 3C), and also in leaf positions 2 and 3 (data not shown). The inhibition of petiole abscission after leaf deblading and ethylene treatment indicates that the petiole AZ cells in the *KD1*-silenced plants were less sensitive to ethylene treatment after auxin depletion. This supports our hypothesis that *KD1* participates in regulating the AZ cell competence to respond to ethylene signals.

The molecular events in the process of tomato pedicel abscission were divided into two phases: early events (0 to 4 h after flower removal) and late events (8 to 14 h after flower removal) [2]. It is important to note that the previous transcriptomic experiment was performed with *Solanum lycopersicum*, cv. 'Shiran', in which 100% of pedicel abscission was obtained already at 14 h after flower removal, and therefore, the time point of 8 h was considered as a late stage. In the present study, we used *Solanum lycopersicum*, cv. 'New Yorker', in which 100% of pedicel abscission was obtained after 20 h (Fig. 3A), and therefore the time point of 8 h is considered as an early stage in the pedicel abscission execution process. The early events (0–4 h) probably lead to acquisition of the competence of FAZ cells to respond to ethylene signaling, and to increased endogenous ethylene biosynthesis. The late events include the execution of pedicel abscission and the development of the defense layer [2].

B. Comparative transcriptome analysis in the FAZ of WT and of *KD1*-silenced plants using a customized AZ-specific array

The microarray experiments were performed with two independently prepared samples, and probes showing at least 2-fold changes in signal intensity were selected for analysis, except for cell wall genes, for which lower intensities were considered. We observed that 64, 76, 96, 111, 304, and 175 transcripts

were upregulated and 120, 126, 319, 58, 397, and 76 transcripts were downregulated in the FAZ samples of the *KD1*-silenced vs WT plants, at 0, 4, 8, 12, 16, and 20 h after flower removal, respectively (Table 1). The complete list of differentially expressed genes was generated and presented in Additional files 1–6: Tables S1-S6. Our microarray results reconfirmed the gene expression profiles reported by Ma et al. [6] in the FAZ at 4 h after flower removal, and revealed the potential role of other regulatory genes associated with *KD1* at the early stage of the pedicel abscission process (Figs. 4–8).

To validate the microarray assay results, we selected the following five genes, *Cellulase1 (Cel1)* (Solyc08g081620 / U13054); *TAPG1* (Solyc02g067630 / U23053); *TAPG4* (Solyc12g096750 / U70481); *KD1* (Solyc06g072480 / AF375969); and *THyPRP* (Solyc07g043000 / X57076), and analyzed their expression by qRT-PCR. The qRT-PCR results were similar to the microarray results (Additional file 7: Figure S1).

C. Mode of action of the inhibition of abscission in *KD1*-silenced plants

The determination of the role of *KD1* in the regulation of abscission in the tomato flower model system, based on the microarray results, should be related to changes in the expression of regulatory genes occurring specifically in the FAZ of the silenced plants at the early stages of pedicel abscission. Therefore, we focused mainly on genes, which showed a modified expression in the FAZ before flower removal (zero time) and at 4 h after flower removal. This early period represents the timing in which the FAZ cells acquire the competence to respond to ethylene signals, which initiates the abscission process [1, 2, 7]. The next abscission phase, the execution stage, which starts during the period of 4 to 8 h after flower removal, can also be regulated both by signals derived from the previous stage, as well as by signals generated during this stage, that cannot be distinguished from one another.

The results presented in Figures 5–9 are organized according to the pattern of gene expression rather than according to the function of the related proteins. Figures 5 and 6 present genes that were downregulated or upregulated, respectively, at zero time; Figures 7 and 8A present genes that were upregulated or downregulated, respectively, at 4 h after flower removal and later on; and Figures 8B and 9 present genes that were transiently upregulated or downregulated, respectively, mainly at 8 h after flower removal. Figures 10 and 11 present genes encoding cell wall degrading enzymes, as well as defense and boundary layer-related proteins, which function in the late abscission execution phases, C and D, and not in the early regulatory events [10, 23].

C1. Genes whose expression was specifically altered in the FAZ of *KD1*-silenced plants at zero time

As a result of the decrease in *KD1* expression in the FAZ of the transgenic plants at zero time (Fig. 1), FAZ-specific changes in the expression of some regulatory genes already occurred at this time (Figs. 4 and 5). Eight genes were downregulated in the transgenic plants at zero time, and their expression remained low up to the end of the experiment (Fig. 4), and 20 genes were upregulated and remained high or gradually decreased later on (Fig. 5). The downregulated genes included: two *MADS-box* genes (Fig. 4A1, A2); a High Mobility Group (HMG) TF (*HMG-type nucleosome assembly factor*) (Fig. 4B); an orthologue gene of the Arabidopsis *Receptor-Like Kinase (RLK)* (Fig. 4C); *Enoyl-CoA Hydratase* (Fig. 4D); *NAD-specific Glutamate Dehydrogenase (GLDH)* (Fig. 4E); and two *Unknown Protein* genes (Fig. 4F1, F2). All the downregulated genes in the FAZ of the silenced plants were not FAZ-specific, and were also significantly expressed in the WT NAZ (Fig. 4), except for *RLK* that was expressed at a low level in the WT NAZ (Fig. 4C).

Twenty genes were specifically upregulated in the FAZ of *KD1*-silenced lines at zero time. These included: Four *F-box family protein* genes (Fig. 5A1-A3). The *Regulatory Particle (RP) RPN3* (Fig. 5A5). Genomic DNA *Chromosome 5P1 clone MBG8* (Fig. 5B1). *Chromodomain Helicase DNA-binding protein4 (CHD4)* (Fig. 5B2). *Histone-lysine N-methyl transferase SETDB1* (Fig. 5B3). Three genes related to ethylene biosynthesis—two *1-Aminocyclopropane-1-Carboxylate Oxidase (ACO)-like proteins* (Fig. 5C1, C2), and one *ACO-homologue* (Fig. 5C3); one *AP2-like Ethylene Responsive Factor (ERF)* (Fig. 5C4). Six *Unknown Protein* genes (Fig. 5D1-D6), a *Serine-Threonine-Protein Phosphatase7* (Fig. 5E), and *Glutaredoxin* (Fig. 5F). All these genes were significantly upregulated in the FAZ of the silenced lines from zero time up to at least 8 h after flower removal (Fig. 5).

Our results revealed that *TAPG::antisense KD1* silencing altered the expression of the following major regulatory genes *specifically* in the FAZ at different levels of regulation: four genes related to plant epigenetics; two *MADS-box* homeobox TFs; eight post translation regulation genes related to ubiquitin-based protein degradation or phosphorylation/de-phosphorylation; and four ethylene-related genes. Some of these altered genes might trigger a cascade of molecular events, leading to alteration of the auxin levels and response in the FAZ at the early stages of the abscission process, resulting in a reduced competence of the FAZ cells to respond to ethylene, as demonstrated in a previous study [6]. Similarly, a reduced competence of the leaf AZ to respond to ethylene after leaf deblading, was also obtained (Figs. 3B, 3C). The four ethylene-related genes, three *ACO* genes (Fig. 5C1-C3) and one *ERF* gene (Fig. 5C4), were unexpectedly upregulated in the FAZ of the silenced plants from zero time up to 20 h. On the other hand, a transiently upregulation of one *ACO* and two *ERF* genes in the WT FAZ was inhibited in the FAZ of the silenced plants (Fig. 6B1-B3). The expression pattern in the WT FAZ and NAZ at different time points of other ethylene-related genes involved in its biosynthesis (*ACSs* and *ACOs*) and perception (*ETRs* and *CTRs*) (data not shown), resembled the previously reported expression pattern for these genes [2]. The expression of these ethylene-related genes as well as the expression of other *ERF* genes, in the FAZ (21 out of 24 *ERFs*) after flower removal, was not affected by *KD1* silencing (data not shown). These data suggest that the inhibition of pedicel abscission induced by *KD1* silencing may not necessarily be ethylene-mediated.

C2. Genes whose expression was specifically altered in the FAZ of *KD1*-silenced plants at 4 h after flower removal

The differentially regulated genes in the FAZ of the silenced plants at 4 h included various regulatory genes at different levels of regulation: epigenetics; TFs; post-translation such as kinase/phosphatase and protein degradation/ubiquitination; transporters; signal transduction; and oxidase/reductase gene families.

The expression of the TF gene encoding the Plant Homeodomain (PHD)-finger family protein, which was specifically upregulated in the WT FAZ at 4 h after flower removal and remained high up to 16 h, was significantly inhibited in the FAZ of the silenced plants (Fig. 6C). The PHD finger protein has a metal binding RING domain (Cys3-His-Cys4) motif. The PHD domain has a conserved Zinc finger (Znf) domain in eukaryotic organisms. PHD finger in proteins related to epigenetics are involved in the interaction between proteins, especially the modification on histone of nucleosome, such as methylation, acetylation, and phosphorylation [24]. A similar expression pattern, i.e. upregulation in the WT FAZ and inhibition in the FAZ of the silenced plants, was also observed for two *bHLH* and *Znf* TF genes, *SibHLH048* and *SibHLH046* (Fig. 6D1, D2). Some *bHLH* and *Znf* TF genes were previously reported to be involved in abscission of olive fruit and tomato flower pedicels [2, 25].

Six genes related to the Ca^{2+} signal transduction, two kinases and four Ca^{2+} /Calmodulin (CaM)-related, which were upregulated at 4 h after flower removal in the WT FAZ, were inhibited in the FAZ of the silenced plants (Fig. 6E1-E4, F1-F2). Calcium ions (Ca^{2+}) serve as a universal messenger involved in the modulation of diverse developmental and adaptive processes in response to various physiological stimuli [26, 27]. Our results showed that the Ca^{2+} /CaM-mediated signal transduction plays a role in regulating the abscission process, and it is probably regulated by *KD1*. Previous reports on various abscission systems demonstrated the involvement and importance of Ca^{2+} /CaM signaling in regulation of organ abscission. Thus, a cycle of water stress/rehydration, which induced citrus leaf abscission, resulted in upregulation of a *CaM* gene in the laminar leaf AZ at 1 h after rehydration [28]. Induction of litchi fruitlet abscission by ethephon treatment resulted in expression changes of genes related to calcium transport and perception. Hence, 19 and 33 transcripts were up- and down-regulated, respectively, following the ethephon treatment, including *CNGC* genes [29]. Similarly, maturation of olive fruits, which induced their abscission, caused upregulation of *CaM*, *CML*, and *Calcium-binding protein kinase* genes in the fruit AZ [25]. Our results demonstrate that in the system of tomato pedicel abscission induced by flower removal *KD1* silencing inhibited the upregulation of Ca^{2+} /CaM signaling-related genes. These findings support the previous reports regarding the involvement of Ca^{2+} /CaM signaling in organ abscission, and suggest that *KD1* plays a significant role in regulating the Ca^{2+} /CaM signal transduction.

Of particular interest is the exocytosis-related gene, *Syntaxin*, which encodes a membrane integrated protein, Q-Soluble N-Ethylmaleimide-sensitive Factor Attachment Protein Receptor (Q-SNARE), necessary for vesicle trafficking. This gene, which was upregulated in the WT FAZ, was significantly downregulated

in the FAZ of the silenced plants (Fig. 6F3). The primary role of SNARE proteins is to mediate vesicle fusion with their target membrane bound compartments participating in exocytosis [30]. Previous reports showed that mobilization of the secretory pathway leads to the release of cell wall modifying enzymes to implement abscission [31, 32]. Additionally, Agusti et al. [28] reported the induction of several genes involved in vesicle trafficking, such as *SNARE-like protein* and *Syntaxin*, in citrus laminar AZ during leaf abscission induced by a cycle of water stress/rehydration. Analysis of gene expression in the melon fruit AZ revealed that a sequential induction of cell wall-degrading genes was associated with the upregulation of genes involved in endo- and exocytosis during mature fruit abscission [33].

Our analysis showed that the *ABC transporter* gene, an ATP-binding cassette transporter which belongs to the ABCA sub-family, was downregulated in the *KD1*-silenced plants compared to the WT (Fig. 6F4). This gene was shown to be involved in auxin transport, and is specifically expressed in the root system [34]. Similarly, the *Coatomer beta subunit* gene was also downregulated in the silenced plants (Fig. 6F1). The coat protein complex genes are responsible for reverse transport of recycled proteins from the Golgi and pre-Golgi compartments back to the ER and vice versa [35].

In the present work, two *Cytochrome P450* genes were downregulated in the FAZ of the *KD1*-silenced plants up to 16 h after flower removal, reaching a similar level of expression to that observed in the WT FAZ at 20 h (Fig. 6G1, G2). Another *Cytochrome P450* gene was also downregulated in the silenced plants up to 12 h after flower removal, and reached a similar level of expression as in the WT FAZ during 12–20 h (Fig. 8D). In Arabidopsis, the cytochrome P450s were shown to be involved in catalyzing the first step of tryptophan-dependent IAA biosynthesis [36]. The involvement of auxin and auxin-related gene expression in pedicel abscission of the WT and the *KD1*-silenced plants was reported previously [6].

Gene expression of several oxidase/reductase-related genes, such as *Laccase* (Fig. 6G3), *Ascorbate Oxidase (AO)* (Fig. 6G5), *Nitric Oxide (NO) reductase* (Fig. 6G6), and *Alcohol Dehydrogenase* (Fig. 6H) was downregulated in the FAZ of the silenced plants, whereas in the WT FAZ these genes were upregulated during the abscission process. This suggests the involvement of oxidative processes in pedicel abscission.

C3. Genes whose expression was specifically- and transiently-altered in the FAZ of *KD1*-silenced plants at 8 h after flower removal

Several genes were specifically and transiently altered in the FAZ of *KD1*-silenced plants at 8 h after flower removal, when the execution of cell separation had already started (22% of pedicel abscission was obtained in the WT—Fig. 3A). One set of genes represents diverse gene families that were transiently upregulated at 8 h in the silenced plants compared to the WT (Fig. 7E-L7). These genes included: A) *CONSTANS interacting protein6* (Fig. 7E), which was shown to control flowering in response to photoperiod in Arabidopsis [37, 38], and leaf induction by cytokinins in tomato [39]. B) *RNA-binding La*

domain protein (Fig. 7F), in which La acts as an RNA polymerase III (RNAP III) TF. *C*) *cDNA clone* (Fig. 7G), that also encodes an RNA-binding protein. *D*) *Dopamine-Monooxygenase N-terminal (DOMON) domain-containing protein*, also called DoH (Fig. 7J), was originally identified in several secreted or cell surface proteins from plants and animals [40]. It is usually associated with other redox domains in larger proteins such as cytochromes b561, and has a suggested capability of transmembrane electron transport [40–42]. *E*) *Seven Unknown Protein* genes that have a similar expression pattern (Fig. 8L1-L7). Since these genes were upregulated in the FAZ of the silenced plants, in which pedicel abscission was significantly inhibited, unlike the WT in which pedicel abscission had already started (Fig. 3A), some of them might have an inhibitory role in the abscission process. Interestingly, seven stress defense-associated genes had the same expression pattern (Fig. 7K1-K7). However, it is not yet clear why these genes were upregulated in the silenced plants, which showed an inhibited abscission phenotype.

A second set of genes, which were specifically and transiently upregulated in the WT FAZ plants at 8 h after flower removal, when abscission had already started, were significantly inhibited in the silenced plants (Fig. 8). These data suggest that part of these genes encode proteins that regulate the abscission execution, including: *A*) *Acid Phosphatase* (Fig. 8A), whose activity was detected in the AZs of various species, such as sour and sweet cherry fruit, bean leaves, and hibiscus pedicels [43–45]. *B*) *Inositol Hexakisphosphate Kinase3* (Fig. 8B1), to which the inositol hexakisphosphate kinase (InsP6) serves as a cofactor that recognizes auxin and the Aux/IAA polypeptide substrate, Transport Inhibitor Response1 (TIR1) [46]. *C*) Four Ca^{+2} /CaM-signal transduction regulatory genes, *SICNGC3* (Fig. 8B2), *CaM-Like protein* (Fig. 9B3), *CaM-Binding protein - SICML35* (Fig. 8B4), and an *AAA-ATPase family protein* (Fig. 8K2). ATPases Associated with diverse cellular Activities (AAA^{+} -ATPases) are AAA-type ATPase-family proteins, which are involved in cellular functions, such as vesicle transport, organelle assembly, membrane dynamics, and protein unfolding [47]. The *Arabidopsis* ATPase Family gene 1, (AFG1)-like protein 1 (AFG1L1), which belongs to the extended superfamily of AAA^{+} -ATPase proteins, binds to CaM in a calcium-dependent manner through a CaM-binding site in its catalytic AAA-domain [48]. The function of these genes and the involvement of Ca^{+2} /CaM in abscission were discussed in details above. Our data indicate that different Ca^{+2} /CaM genes regulate early and late events in the abscission process. *D*) Different transporter-related genes, such as *Glycosyltransferase (GTF)* (Fig. 8C1), *UDP-Glycosyltransferase* (Fig. 8C2), three *ABC/ABC-2* type transporters (Fig. 8E1-E3), an *Amino Acid Transporter* (Fig. 9E4), and *Vesicular Glutamate Transporter3 (VGULT3)* (Fig. 8E5). *E*) Two decarboxylase genes, *AADC1A* (Fig. 8F1) and *Decarboxylase Family Protein* (Fig. 8F2). *F*) Amino acid biosynthesis/metabolism genes - *Glutamine Amido Transferase (GATase)* (Fig. 8G1) and *WPP Domain-Associated Protein* (Fig. 8G2). The *Arabidopsis* WPP-domain proteins are developmentally associated with the nuclear envelope and promote cell division [49]. *G*) *Cysteine Desulfurase1 (DES1)* (Fig. 8H), which encodes the enzyme L-cysteine desulfhydrase that catalyzes the desulfuration of L-cysteine. The GFP fused to the *DES1* promoter was reported to be highly expressed in the AZ of seeds and siliques, and the *des1* mutants exhibited an altered leaf senescence phenotype [50]. *H*) The gibberellin (GA) receptor gene, *GID1L2*, which was upregulated in the WT FAZ at 4 h and peaked at 8 h after flower removal (Fig. 8I),

suggesting that the inhibition of abscission by *KD1* silencing might be mediated also through the inhibition of *GIDIL2.1*) Some other *Unknown Protein* genes (Fig. 8L1-L4).

D. *KD1* silencing decreases the expression of cell wall modifying genes

It is well established that cell wall loosening and cell separation occurring in the AZ are controlled by distinct sets of cell wall degrading enzymes. Dissolution of the middle lamella or the shared cell wall in the AZ is a fundamental step in the abscission process. Enzymes and proteins associated with disassembly and modification of the cell wall include PGs, cellulases, endoglucanases, pectin methylesterases, pectate lyases, xyloglucan endotransglucosylase/ hydrolases (XTH), and expansins (EXP) [10, 51–54]. Therefore, the expression pattern of these cell wall modifying enzymes occurring at the late stages of abscission, was used as an additional marker for confirming the effects of *KD1* silencing in delaying pedicel abscission.

Our transcriptome analysis revealed a specific upregulation of 28 genes encoding cell wall modifying enzymes in the WT FAZ, whereas in the NAZ the expression of most of them did not change and remained very low during the entire experimental period (Fig. 9A-J). These genes, which belong to 11 families, were downregulated by *KD1* silencing, as manifested by their lower or delayed expression in the FAZ of the silenced plants (Fig. 9). Only *Cel8*, *XTH26*, and *EXP2* genes exhibited some increased expression in the NAZ (Fig. 9B3, C7 and F1). Among the cell wall hydrolyzing enzymes, *TAPG4,5*, and *XTH3a,3c,3d,7,10* were upregulated in the WT FAZ at 4 h after flower removal, and their expression remained high up to 16 h (Fig. 9A3, A4, C1-C5). On the other hand, *Glucanase*, *Pectin Esterase*, and *EXP* genes were upregulated only at 8–12 h after flower removal (Fig. 9E1–J). The tomato AZ-specific *TAPG* genes, *TAPG1,2,4,5*, were downregulated in the silenced plants compared to the WT (Fig. 9A1-A4). The expression patterns of *TAPG1,2,4* in the WT FAZ were identical to their patterns reported previously [2], thereby confirming the microarray results obtained by the tomato AZ-specific microarray chip. Our results further demonstrate that the organ abscission execution, manifested by upregulation of cell wall degrading proteins at phase C of the abscission process [10, 53–55], is a programmed event, in which these proteins were sequentially increased (Fig. 9).

D1. *KD1*

Phase D of the abscission process, in which the production of a protective defense layer occurs [10, 53–55], was shown to significantly overlap with the execution phase C of abscission [23]. We present here only selected data of the numerous defense genes that were specifically upregulated

in the tomato WT FAZ, and were significantly inhibited in the *KD1*-silenced plants. Of the set of genes that were specifically and transiently altered in the WT FAZ at 8 h after flower removal, three genes should be mentioned: *Omega-6 Fatty Acid Desaturase* (Fig. 8J1) and two *Lipoxygenase (LOX)* genes, *Cevi34/LOX* (Fig. 8J2) and *LOX* (Fig. 8J3). Some of the defense-related genes were upregulated in the WT FAZ very early after flower removal (Fig. 10A-E2; marked with red*), while others were upregulated only later on (Fig. 10E3, E4, F1, F2; marked with green*). The expression of all these genes was inhibited in the *KD1*-silenced plants (Fig. 10). These genes are related to abscission, as they were upregulated only in the WT FAZ, in which abscission took place after flower removal. These results confirm the overlapping between phases C and D of the abscission process.

Conclusions

Based on the results presented in this study, we could further elucidate how *KD1* delays pedicel abscission. The present data indicate that *KD1* is a master regulator of tomato pedicel abscission, as the expression of important regulatory genes was modified in the FAZ of the *KD1*-silenced plants at 4 h after flower removal, i.e. at the early stage of the abscission process, compared to the WT FAZ. This led to the inhibition of pedicel abscission, as summarized in Figure 11. Moreover, it should be noted that *KD1* expression decreased in the silenced plants already at zero time, which led to the modified expression of regulatory genes before flower removal (Fig. 11A). These early modified regulatory genes included epigenetic modifiers, TFs, post translation regulators, and antioxidative defense factors. Some of them might directly affect auxin homeostasis and transport, resulting in slowing down auxin depletion in the FAZ, which leads to inhibition of the acquisition of the competence of the FAZ to respond to the ethylene signaling. The changes in gene expression occurring at 4 h after flower removal (Fig. 11B) might result either from a direct effect of *KD1* silencing, or from the changes in gene expression occurring at zero time, prior to abscission induction (Fig. 11A). The above data suggest a possible mode of action for the manipulation of auxin levels and response obtained following *KD1* silencing, which finally leads to inhibition of pedicel abscission.

The effects of *KD1* silencing, which result in the inhibited abscission phenotype, include also indirect effects leading to downregulation of genes involved in abscission execution and defense. Thus, the inhibited expression of exocytosis and protein transporters such as AAA-ATPase and Syntaxin, which inhibited the export of Golgi-derived vesicles containing cell wall degrading enzymes across the plasma membrane of AZ cells, might have an important role in the inhibited abscission phenotype of the *KD1*-silenced plants. It seems therefore, that the inhibitory effect of *KD1* silencing on flower pedicel abscission is not limited to manipulation of auxin levels and response as previously reported, but it probably also operates via alteration of other regulatory pathways that delay the acquisition of the competence of the FAZ cells to respond to ethylene signaling and abscission execution (Fig. 11B).

Since *KD1* belongs to the class I KNOX family, its role in organ abscission is in agreement with the various regulatory roles of class I KNOX family proteins, which were found to regulate several genes involved in biosynthesis and signal transduction of various plant hormones, TFs, and lignin synthesis. Taken together, the present study shades light on the role of *KD1* as a master regulator of various signaling factors and regulatory genes involved in organ abscission processes, in addition to its well-documented involvement in leaf development, shoot apical meristem maintenance, and differentiation of flower meristems.

Methods

Plant material and abscission induction treatments

The experiments were performed with tomato *Solanum lycopersicum*, cv. 'New Yorker') plants, grown from seeds obtained from the Tomato Genetics Resource Center, University of California, Davis, USA. All plants were grown in a greenhouse located in The Volcani Center, Israel, under a controlled temperature of 25°C and natural daylight. The flower abscission experiments were carried out with plants grown in 10-L pots. The inflorescences of both WT and the *KD1*-antisense transgenic lines were harvested from 4-month-old plants as previously described [2, 7]. The experiment protocols and pedicel abscission assays were performed in a controlled observation room maintained at 20°C and 60 to 70% relative humidity with continuous light of 14 $\mu\text{mol m}^{-2} \text{s}^{-1}$, as previously described [2, 7, 23]. The number of abscised pedicels was monitored daily by touching the distal side of the FAZ, for calculating the accumulated percentage of pedicel abscission.

Leaf abscission experiments were carried out with plants grown in 576 cm^3 pots, which had 6 to 8 true leaves per plant. For abscission induction, the leaves of the WT and the *KD1*-silenced transgenic plants were debladed for auxin depletion, and 48 h after leaf deblading, the plants were treated with 10 $\mu\text{L L}^{-1}$ ethylene for 24 h at 23°C, as previously described [22]. After the ethylene treatment, the plants were transferred to a controlled observation room, and the accumulated percentage of petiole abscission was monitored at specified time intervals (during days 1 to 10) for each true leaf at four positions above the cotyledons. The kinetics of flower and leaf abscission was determined in three different experiments,

using 10–12 plants for each line, with similar results, and the results of one representative experiment are presented.

Vector construction and plant transformation

The generation of the *TAPG4::antisense KD1* transgenic plants was basically performed as previously described [6]. Briefly, a 2379-bp fragment of the AZ-specific *TAPG4* promoter from tomato genomic DNA and a 227-bp fragment of the *KD1* gene from tomato cDNA were amplified, and then sub-cloned into the modified binary vector GSA1285 in an antisense orientation. We used the primers 5'-TCTCAGCTCAGTGAACCTCATGG-3' and 5'-TTGTGGCAATCTAGCCA-TACAT-3' for the *KD1* amplification, introduced the plasmid constructs into *Agrobacterium tumefaciens* (LBA4404), and transformed the tomato 'New Yorker' as previously described [56]. In this way we generated 10 independent transgenic lines, from which we selected two representative transgenic lines (lines A and E, T4 generation) as previously reported [6], for further analysis.

Gene expression profiling

For expression and microarray studies, we used samples of the FAZ and the NAZ of the WT and the FAZ of *TAPG4::antisense KD1* line E, collected in duplicates at various time points (0, 4, 8, 12, 16, and 20 h after flower removal). The RNA was extracted, treated with DNase, processed and checked for its quality, using the Agilent platform (Agilent, Palo Alto, CA), as previously described [7, 22].

Microarray

The experimental procedures for microarray labeling, hybridization, and scanning were performed as previously detailed [7], using the AZ-specific microarray chip, AMADID: 043310 (Genotypic Technology, Pvt. Ltd, Bangalore, India) for hybridization of the labeled cRNA samples [22]. We used the Feature Extraction software of Agilent V-11.5 for the data extraction from images. The analysis of the microarray data, using duplicate samples for each time point, to identify significant genes that were up- and down-regulated within the group of samples, was basically performed as previously detailed [7].

Validation of gene expression by qRT-PCR

All the procedures for validation of gene expression were performed as previously detailed [7], according to the primers sequences, amplicon length, and annealing (T_m) presented in Additional file 8: Table S7. These primers matched the microarray probes, and the qRT-PCR and microarray analyses were performed with the same RNA samples, as previously described [22]. *ACTIN* was used as the reference gene for determination of the relative expression levels of the identified genes.

Sequence deposition

The data for the *TAPG::antisense KD1* FAZ samples (12 arrays) were submitted under the Gene Expression Omnibus database NCBI-GEO accession id: GSE64564. The microarray data for WT (cv. 'New Yorker') FAZ and NAZ samples (12 arrays each) were submitted as previously detailed [7] under accession id: GSE64221. The data will be released for public access upon acceptance of the manuscript.

List Of Abbreviations

AAA⁺-ATPases: ATPases Associated with various cellular Activities; ACC: 1-Amino-cyclopropane-1-carboxylic acid; ACO: ACC oxidase; AZ: Abscission zone; CaM: Calmodulin; ERF: Ethylene responsive factor; FAZ: Flower AZ; GA: Gibberellin; GO: Gene ontology; KD1: Knotted1-like Homeobox protein; NAZ: Non-AZ; RNA-Seq: RNA sequencing; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; TAPG4: Tomato abscission polygalacturonase4; TF: Transcription factor; WT: Wild type; XTH: Xyloglucan endotransglucosylase/hydrolase.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its Additional files. Any other information is available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SS and SM conceived the original screening and research plans. SM, SPH, and JR supervised the experiments. SS performed most of the experiments and analyzed the data. CM and CZJ prepared the constructs and produced the silenced plants. BK and ShS provided technical assistance to SS. SM and SPH conceived the project and wrote the article with contributions of all the authors. JR and MSR supervised, critically revised and complemented the writing. All authors have read and approved the final version of the manuscript.

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Tables

Table 1: Transcriptome responses of the FAZ tissues at various time points after flower removal in the transgenic (*TAPG4::antisense KD1*) line compared to the WT. The data represent the total numbers of differentially expressed transcripts (fold changes: downregulated ≤ -2 ; upregulated ≥ 2) at different time points after flower removal during the abscission process.

Time after flower removal (h)	Number of differentially expressed transcripts in the FAZs of <i>TAPG4::antisense KD1</i> vs WT plants	
	Upregulated	Downregulated
0	64	120
4	76	126
8	96	319
12	111	58
16	304	307
20	175	76

Figures

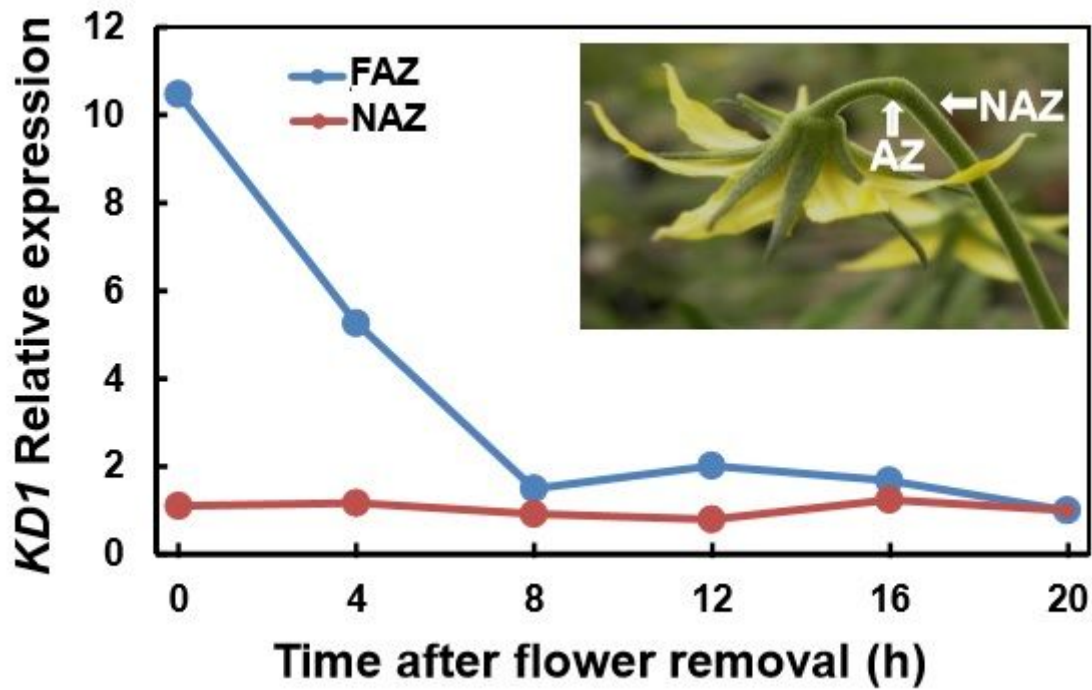


Figure 1

Effect of flower removal on the expression of KD1 in the WT FAZ and NAZ. The KD1 expression was determined by qRT-PCR at 0, 4, 8, 12, 16, and 20 h after flower removal. The relative quantification of the gene expression level by the qPCR assay was determined by the comparative CT method $2^{-\Delta\Delta CT}$, using Actin as a reference gene. The ΔCT values were compared to the 20-h ΔCT value for KD1 to generate $2^{-\Delta\Delta CT}$ values. The results are means of three biological replicates \pm SD. Inset, localization of the FAZ and NAZ along the flower pedicel, from which tissue samples were taken.

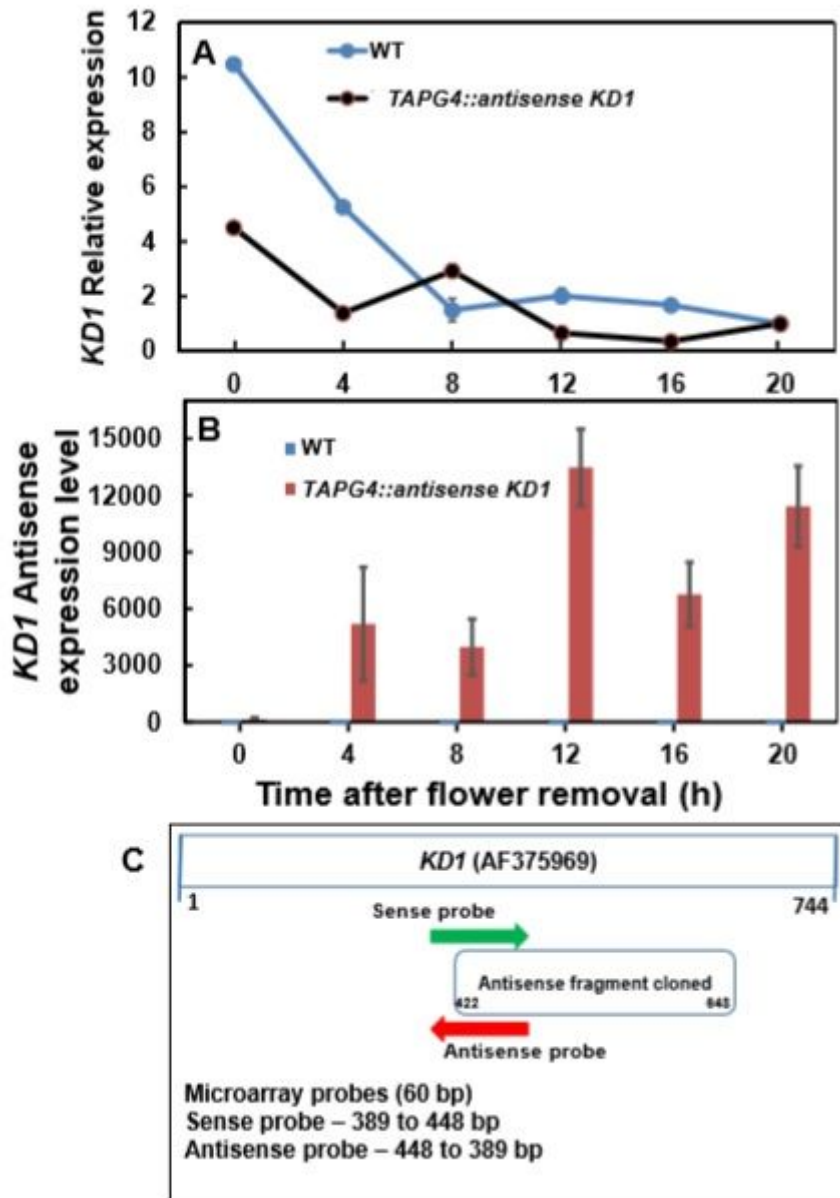


Figure 2

Effect of flower removal on the qRT-PCR expression levels of KD1 (A) and on the array-measured expression levels of the antisense probe of KD1 (B) in the FAZs of the WT and TAPG4::antisense KD1 - line E. The schematic illustration of the sense and antisense microarray probes spanning the KD1 gene is presented (C). The qRT-PCR assay was performed as detailed in Figure 1. The fragment (422-648th bp) of the KD1 gene (AF375969) was cloned for antisense into the vector. The sense probe from the microarray spans the region of 389-448th bp, and the antisense probe from the microarray spans the region of 448-389th bp.

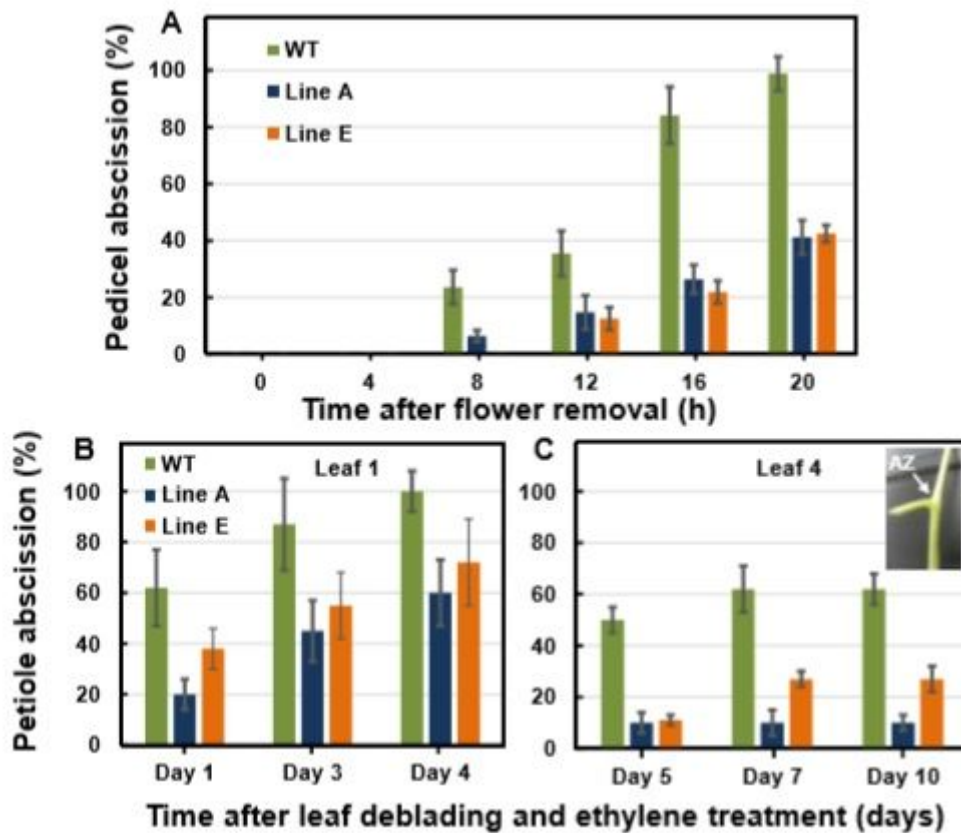


Figure 3

Effects of TAPG4::antisense KD1 silencing on the abscission kinetics of flower pedicels (A) and petioles of leaf 1 (B) and leaf 4 (C). The accumulated percentage of pedicel abscission was monitored at 0, 4, 8, 12, 16, and 20 h following flower removal. Petiole abscission was induced in intact plants by leaf deblading followed by treatment with 10 μ L L-1 ethylene for 24 h applied 48 h after leaf deblading. The accumulated percentage of petiole abscission was monitored during days 1 to 10 for true leaf positions 1 and 4 above the cotyledons. The results are means of three experiments \pm SD (n = 40 explants for flowers and at least 10 plants for leaves in each experiment). C - Inset, the location of the leaf AZ is indicated by the white arrow.

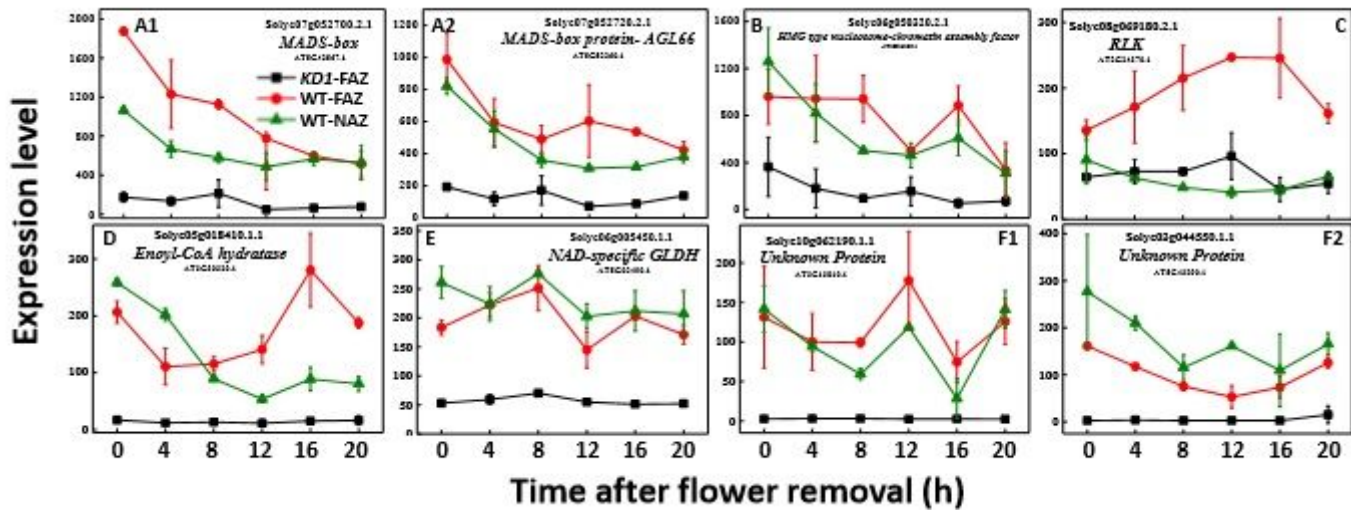


Figure 4

Effect of antisense silencing of KD1 on the kinetics in array-measured expression levels of genes that were specifically downregulated in the FAZ of TAPG4::antisense KD1-silenced plants, before (at zero time) and after flower removal compared to the FAZ of WT plants. TAPG4::antisense KD1 silenced line E was used. Expression levels were measured for the following genes: MADS-box transcription factor1 (A1) and MADS-box protein-AGL66 (A2); High Mobility Group (HMG) type nucleosome/chromatin assembly factor (B); Receptor-Like protein Kinase (RLK) (C); Enoyl-CoA Hdratase (D); NAD-specific Glutamate Dehydrogenase (GLDH) (E); and Unknown Protein genes (F1, F2). Transcript identities are indicated in the graphs by their gene ID and their Arabidopsis (At) gene number and/or their nucleotide accession number. The results are means of two biological replicates \pm SD. The Red and Green lines represent the gene expression in the FAZ and NAZ of the WT plants, and the Black lines represent the gene expression in the FAZ of KD1-silenced plants, respectively.

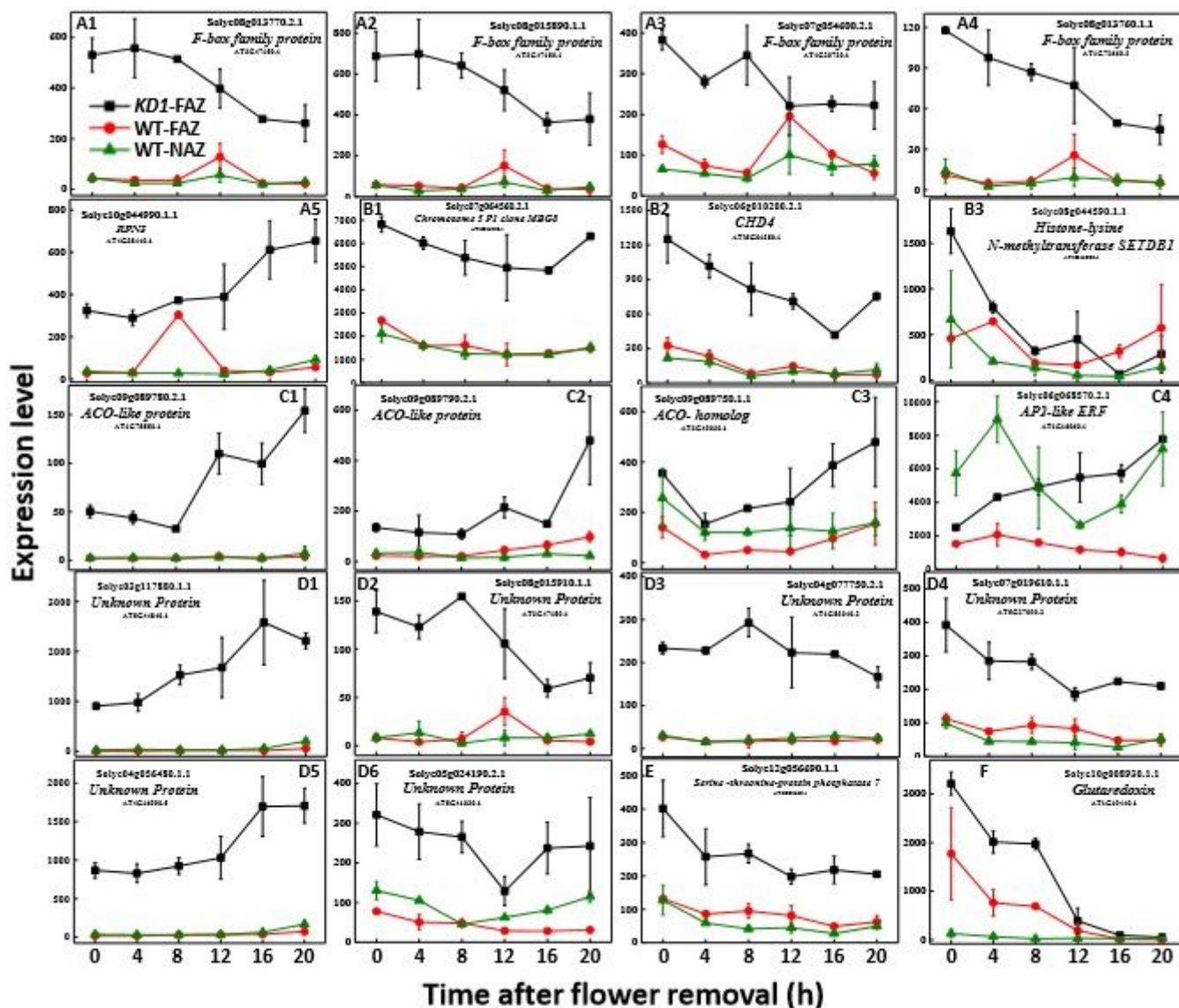


Figure 5

Effect of antisense silencing of KD1 on the kinetics in array-measured expression levels of genes that were specifically upregulated in the FAZ of TAPG4::antisense KD1-silenced plants, before (at zero time) and after flower removal, compared to the FAZ of WT plants. Expression levels were measured for the following genes: F-box family proteins (A1-A4), and 26S proteasome non-ATPase regulatory subunit3 (RPN3) (A5); genomic DNA chromosome 5P1 clone MBG8 (B1); Chromodomain Helicase DNA-Binding protein4 (CHD4) (B2), and Histone-lysine N-methyltransferase SETDB1 (B3); two 1-Aminocyclopropane-1-Carboxylate Oxidase (ACO)-like protein genes (C1, C2), ACO-homologue (C3), and AP2-like-Ethylene Response Factor (ERF) (C4); six Unknown Protein genes (D1-D6); Serine-threonine-protein phosphatase7 (E); and Glutaredoxin (F). All other details are as described in Figure 4.

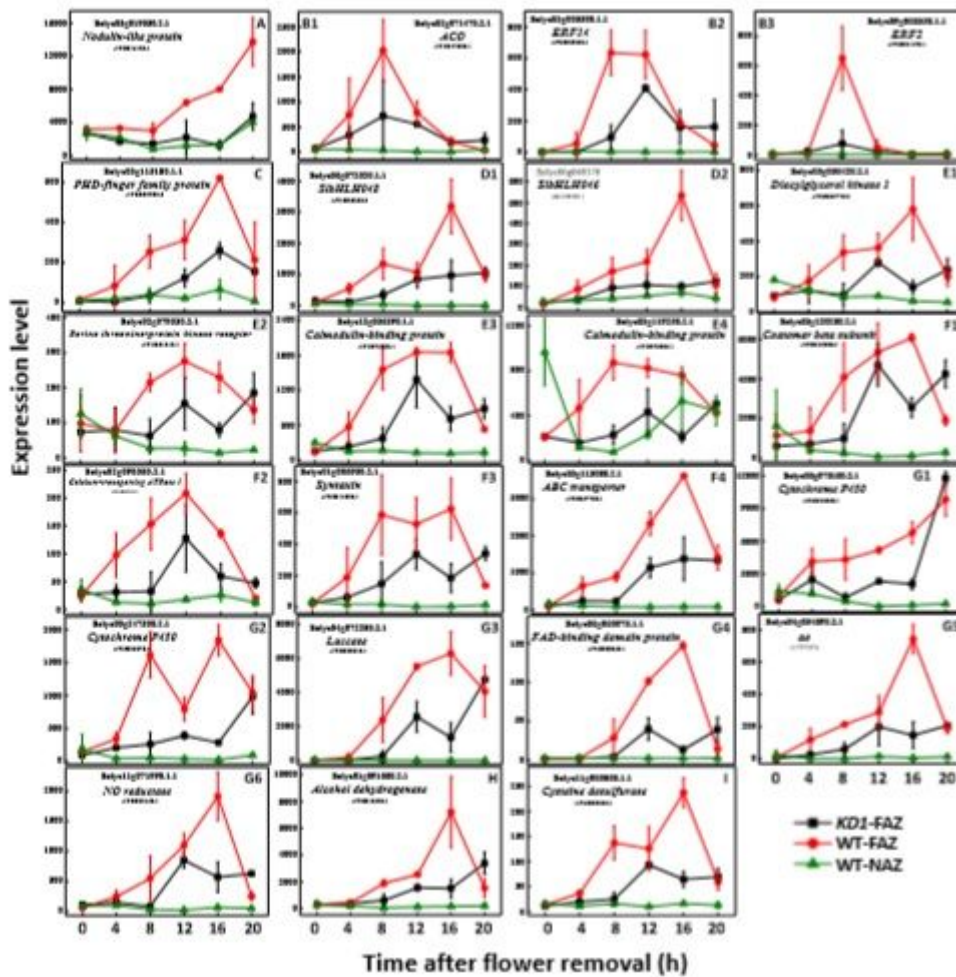


Figure 6

Effect of antisense silencing of KD1 on the kinetics in array-measured expression levels of genes that were specifically modified in the WT FAZ plants at 4 or 8 h after flower removal. Expression levels were measured for the following genes: Nodulin-like protein (A); 1-Aminocyclopropane-1-Carboxylate Oxidase1 (ACO1) (B1); Ethylene-Responsive Factor14 (ERF14) (B2); ERF2 (B3); PHD-finger family protein (C); *Solanum lycopersicum* Basic Helix-Loop-Helix048 (SlbHLH048) (D1), SlbHLH046 (D2); Diacylglycerol Kinase1 (E1); Serine/threonine-protein kinase receptor (E2); two Calmodulin-Binding protein genes (E3, E4); Coatomer beta subunit (F1), Calcium-transporting ATPase1 (F2), Syntaxin (F3), and ABC transporter (F4); two Cytochrome P450 genes (G1, G2), Laccase (G3), FAD-binding domain-protein (G4), Ascorbate Oxidase (AO) (G5), and Nitric Oxide (NO) reductase (G6); Alcohol Dehydrogenase (H); and Cysteine Desulfurase (I). All other details are as described in Figure 4.

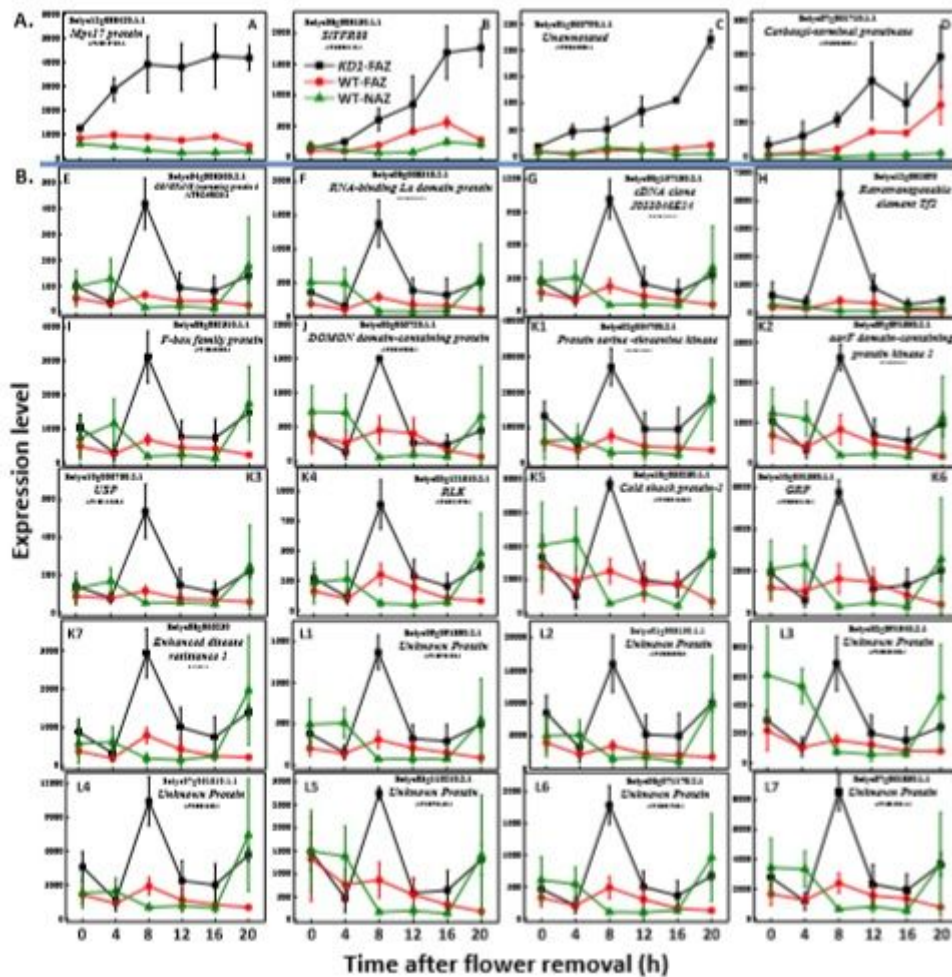


Figure 7

Effect of antisense silencing of KD1 on the kinetics in array-measured expression levels of genes that were specifically continuously (A-D) or transiently (E-L7) upregulated between 0 to 20 h or at 8 h after flower removal, respectively, only in the FAZ of TAPG::antisense KD1-silenced plants. Expression levels were measured for the following genes: MpV17 protein (A); SITFR88 (B); Unannotated (C); Carboxyl-terminal proteinase (D); CONSTANS interacting protein6 (E); RNA-binding La domain protein (F); cDNA clone (G); Retrotransposable element Tf2 (H); F-box family protein (I); DOMON domain-containing protein (J); Protein serine-threonine kinase (K1), aaF domain-containing protein kinase1(K2), Universal Stress Protein (USP) (K3), Receptor-Like Kinase (RLK) (K4), Cold Shock protein-1 (K5), Glycine-rich RNA-binding Protein (GRP) (K6), and Enhanced Disease Resistance1 (K7); and seven Unknown Proteins genes (L1-L7). All other details are as described in Figure 4.

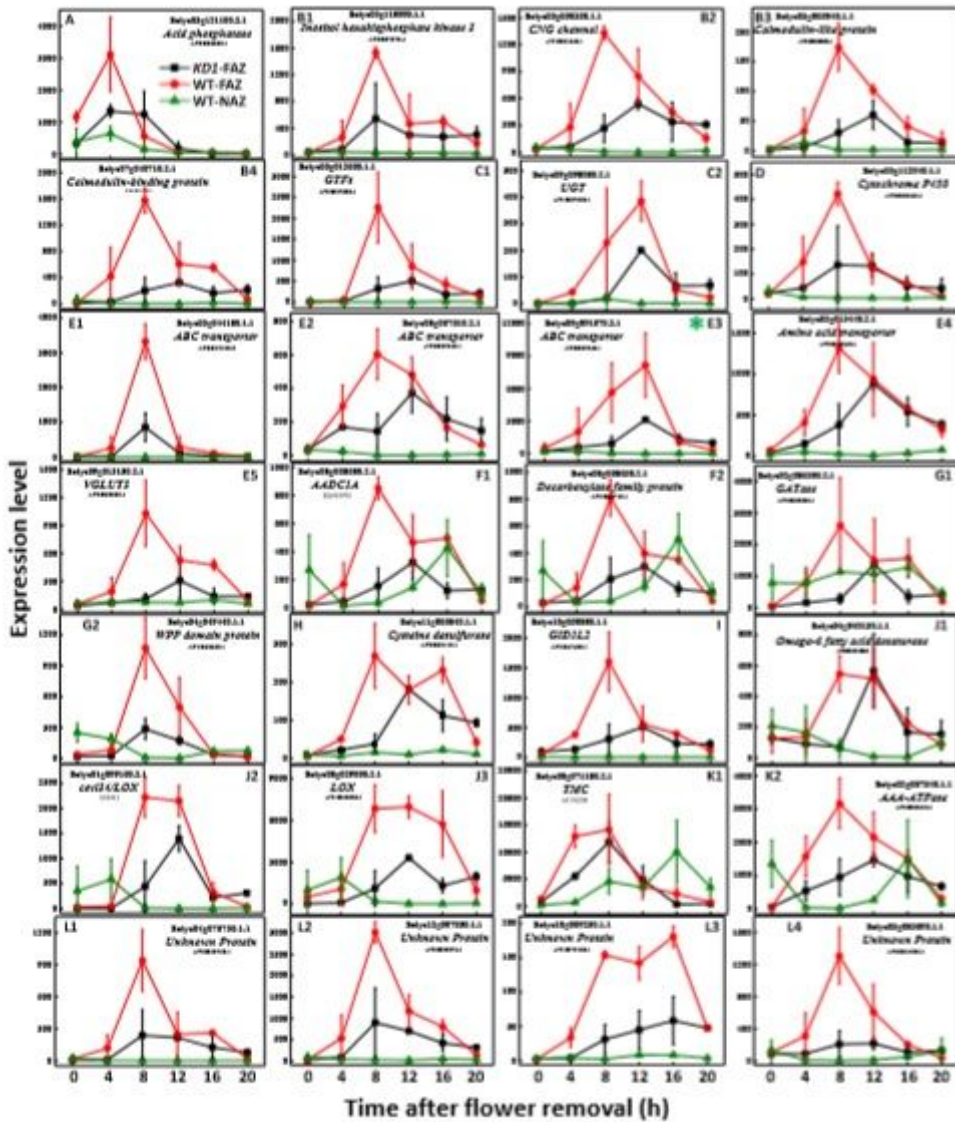


Figure 8

Effect of antisense silencing of KD1 on the kinetics in array-measured expression levels of genes that were specifically and transiently upregulated in the WT FAZ plants, peaking at 4, 8 or 12 h after flower removal. Expression levels were measured for the following genes: Acid phosphatase (A); Inositol hexakisphosphate kinase3 (B1), Cyclic Nucleotide Gated channel (CNG channels) (B2), Calmodulin-like protein (B3), and Calmodulin-Binding protein (B4); Glycosyltransferases (GFTs) (C1), and UDP-Glucosyl Transferase (UGT) (C2); Cytochromes P450 (D); three ABC transporter family protein genes (E1-E3), Amino acid transporter protein (E4), and Vesicular Glutamate Transporter3 (VGLUT3) (E5); Aromatic Amino acid Decarboxylase1A (AADC1A) (F1), and Decarboxylase family protein (F2); Glutamine Amido Transferases (GATase) (G1), and Tryptophan-Proline-Proline (WPP) domain (G2); Cysteine Desulfurase (H); Gibberellin receptor GID1L2 (I); Omega-6 fatty acid desaturase (J1), cevi34/Lipoxygenase (LOX) (J2), LOX (J3); Multicystatin (TMC) (K1), and AAA-ATPase (K2); and four Unknown Protein genes (L1-L4). All other details are as described in Figure 4.

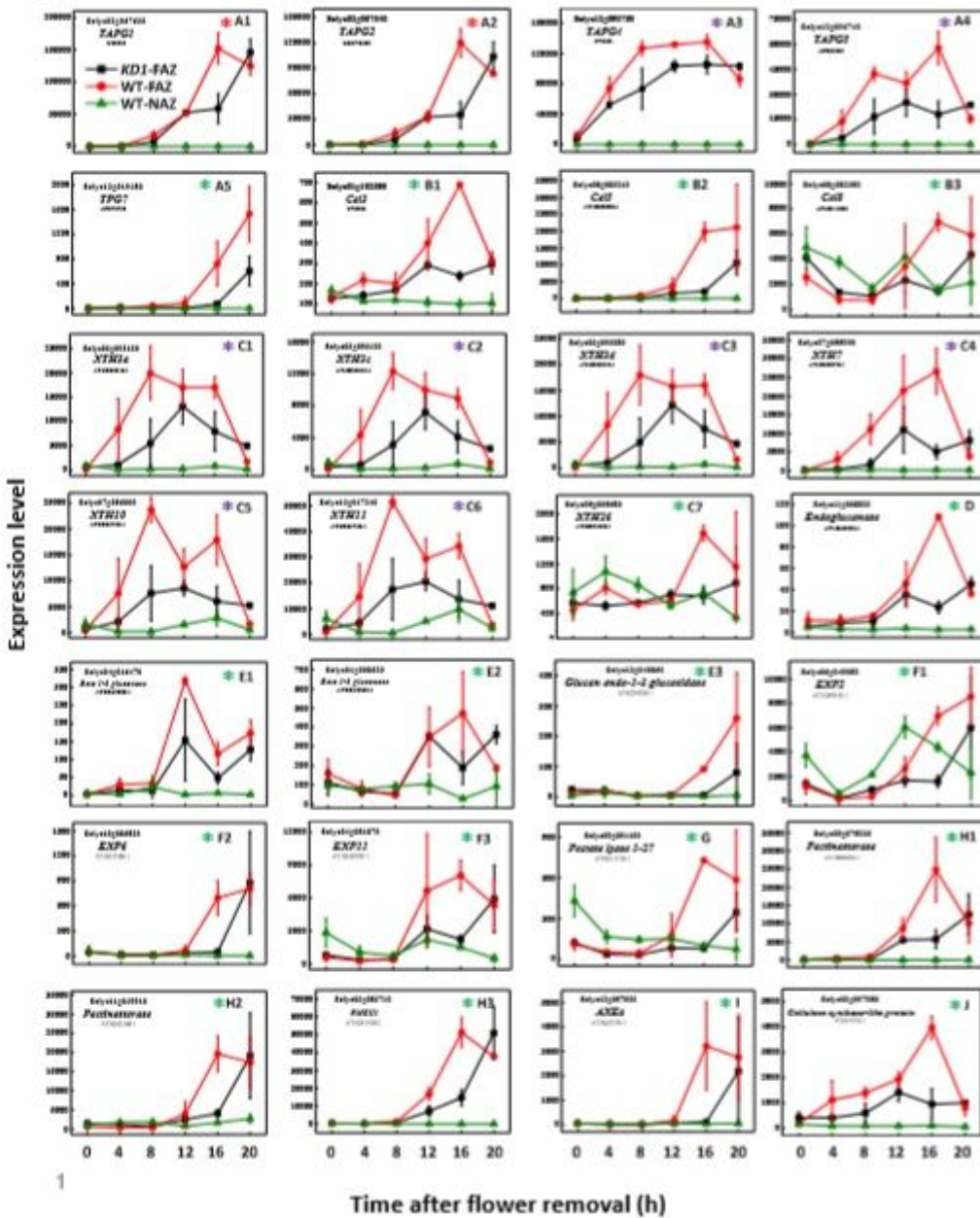


Figure 9

Effect of antisense silencing of KD1 on the kinetics in array-measured expression levels of genes encoding cell wall modifying enzymes that were specifically upregulated in the WT FAZ at specific time points after flower removal: early - 4 (*) or 8 (*) h or late 12-20 (*) h. Expression levels were measured for the following genes: five Tomato Abscission Polygalacturonase (TAPG) genes (A1-A5); three Cellulase (Cel) genes (B1-B3); seven Xyloglucan endo Transglucosylase/ Hydrolase (XTHs) genes (C1-C7); Endoglucanase (D); three β -1,3-glucanase genes (E1-E3); three Expansin (EXP) genes (F1-F3); Pectate Lyase 1-27 (G); two Pectinesterase genes (H1-H2), and Pectinesterase/pectinesterase inhibitor U1 (PMEU1) (H3); Acetyl Xylan Esterase a (AXEa) (I); and Cellulose Synthase-Like protein (J). All other details are as described in Figure 4.

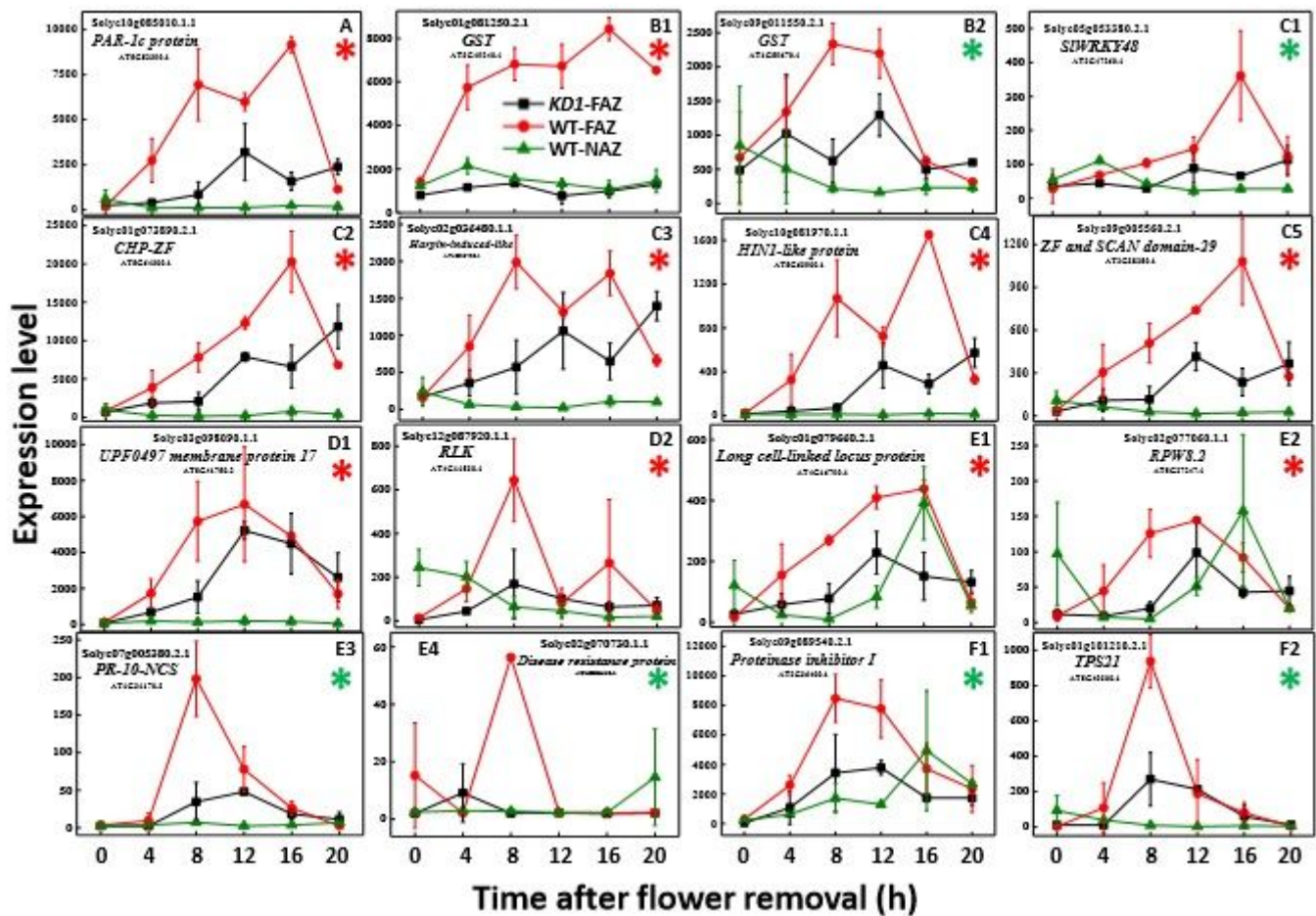


Figure 10

Effect of antisense silencing of KD1 on the kinetics in array-measured expression levels of genes specifically transiently upregulated in the WT FAZ plants at the early stage of abscission, 4 (*) or 8 (*) h after flower removal. Expression levels were measured for the following genes: Photo Assimilate-Responsive1c protein (PAR-1) (A); two Glutathione S-Transferase protein (GST) genes (B1, B2); Solanum lycopersicum SIWRKY48 (C1), Cysteine/Histidine-rich Zinc Finger protein-like (CHP-ZF) (C2), Harpin-Induced-Like (C3), and HIN1-like protein (C4); ZF and SCAN domain-29 (ZSCAN29) (C5); UPF0497 membrane protein17 (D1), and Receptor-Like Kinase (RLK) (D2); Long cell-linked locus protein (E1), Resistance to Powdery Mildew8 (RPW8.2) (E2), PR-10-related Norcochloraurine Synthase-like protein (PR-10-NCS) (E3), and Disease Resistance protein (E4); Proteinase InhibitorI (F1), and α -humulene/(-)-(E)- β -Caryophyllene Synthase (TPS21) (F2). All other details are as described in Figure 4.

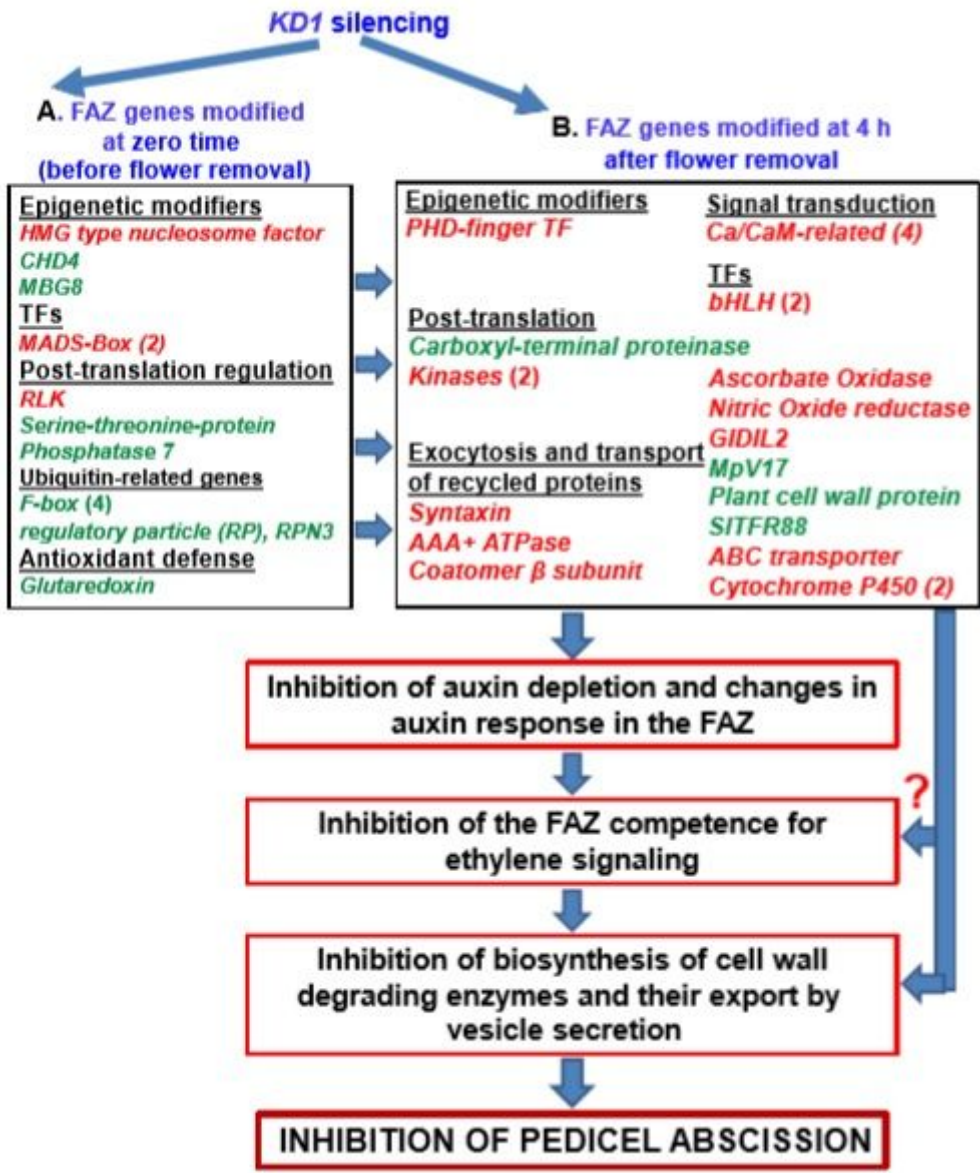


Figure 11

Summary of the genes with altered expression in the FAZ of TAPG4::antisense KD1 plants before (at zero time) and at 4 h after flower removal in response to KD1 downregulation and abscission induction by flower removal. The altered gene expression led to a decrease of the postulated events resulting in the inhibition of pedicel abscission. Inhibition of auxin depletion and changes in the auxin response in the FAZ at the early abscission stages were demonstrated by Ma et al. [6]. Genes that were specifically upregulated or downregulated in the FAZ of KD1-silenced plants compared to the WT are marked in Green or Red, respectively. The numbers listed besides the gene names indicate the number of genes in the gene family that were affected by KD1 silencing.

Supplementary Files

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