Ethylene-dependent and -independent pathways controlling floral abscission are revealed to converge using promoter::reporter gene constructs in the ida abscission mutant

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Abstract

The process of floral organ abscission in Arabidopsis thaliana can be modulated by ethylene and involves numerous genes contributing to cell separation. One gene that is absolutely required for abscission is INFLORESCENCE DEFICIENT IN ABSCISSION, IDA, as the ida mutant is completely blocked in abscission. To elucidate the genetic pathways regulating floral abscission, molecular markers expressed in the floral abscission zone have been studied in an ida mutant background. Using plants with promoter–reporter gene constructs including promoters of a novel FLORAL ABSCISSION ASSOCIATED gene (FAA) encoding a putative single-stranded binding protein (BASIL), chitinase (CHIT::GUS) and cellulase (BAC::GUS), it is shown that IDA acts in the last steps of the abscission process. These markers, as well as HAESA, encoding a receptor-like kinase, were unaffected in their temporal expression patterns in ida compared with wild-type plants; thus showing that different regulatory pathways are active in the abscission process. In contrast to BASIL, CHIT::GUS and BAC::GUS showed, however, much weaker induction of expression in an ida background, consistent with a reduction in pathogen-associated responses and a lack of total dissolution of cell walls in the mutant. IDA, encoding a putative secreted peptide ligand, and HAESA appeared to have identical patterns of expression in floral abscission zones. Lastly, to address the role of ethylene, IDA::GUS expression in the wild type and the ethylene-insensitive mutant etr1-1 was compared. Similar temporal patterns, yet restricted spatial expression patterns were observed in etr1-1, suggesting that the pathways regulated by IDA and by ethylene act in parallel, but are, to some degree, interdependent.

Key words: Cellulase, chitinase, HAESA, floral abscission associated gene, wounding.

Introduction

Abscission, the shedding of entire organs during the course of normal plant development is an essential mechanism that plants have developed to discard organs that no longer serve essential functions to the plant, and to respond to environmental events such as disease or pathogen challenge (Patterson and Bleecker, 2004). Arabidopsis has a developmentally determined programme of floral organ abscission (Bleecker and Patterson, 1997). The process takes place at predetermined positions called abscission zones (AZs), at the bases of the filaments, petals, and sepals (Bleecker and Patterson, 1997). In the wild type the abscission process can be divided into several steps (Patterson, 2001). Separation results from catabolic alterations in the middle lamellae and the primary cell wall (Sexton and Roberts, 1982). The enzymatic machinery responsible for the disassembly and modification of the cell-wall components include enzymes such as endo-β-(1,4)-glucanases (cellulases) (Lewis and Varner, 1970; del Campillo et al., 1990), polygalacturonases (Kalaitzis et al., 1997; Roberts et al., 2000),...
pectinases, and expansins (Cho and Cosgrove, 2000). In addition, some defence genes, such as chitinase (Volk et al., 1998) and jasmonic acid biosynthesis enzymes (Kubistelting et al., 1999), have been observed to be up-regulated in AZs (Patterson, 2001) to protect the plant from pathogen invasion. The involvement of these enzymes in abscission has made the genes encoding them valuable tools to track the abscission process in wild-type plants, and to enable comparison of the process in mutant backgrounds.

The concept that AZ cells are targeted for specific inter- and intracellular signalling events is well established (Roberts et al., 2002), and various growth regulators have been implicated in triggering the AZ cells to express genes necessary for cell wall hydrolysis. Evidence from many different abscission model systems support ethylene’s promoting and auxin’s inhibiting role in the regulation of abscission in dicotyledonous plants (Addicott, 1982). In Arabidopsis, exposure to exogenous ethylene accelerates the abscission process and induces corolla senescence (Bleecker and Patterson, 1997; Butenko et al., 2003). The mechanism leading to the increased sensitivity to ethylene prior to abscission is unknown, but the ethylene receptor ETR1 is likely to be involved (Schaller and Bleecker, 1998). Antisense lines for HAP1 (Bleecker and Patterson, 1997; Patterson and Bleecker, 2004), and RNA interference plants exhibiting reduced expression of the MADS domain factor AGL15 (Fernandez et al., 2000), the delayed floral organ abscission (dab) mutants (Patterson and Bleecker, 2004), and RNA interference plants exhibiting reduced levels of the actin related protein ARP7 (Kandasamy et al., 2005).

The authors have earlier reported on the identification of the floral abscission mutant inflorescence deficient in abscission (ida) which retains its floral parts indefinitely (Butenko et al., 2003). The ida mutant is ethylene-sensitive and perfectly capable of responding to exogenous ethylene at a whole plant level, but the mutant’s deficiency in floral abscission can not be rescued by exposing plants to saturating levels of ethylene gas (Butenko et al., 2003). When IDA is overexpressed using the 35S promoter, floral abscission takes place earlier, and abscission is also seen in vestigial abscission zones (Stenvik et al., 2006). To elucidate the abscission pathways where IDA might be involved, promoter β-glucuronidase (GUS) expression studies in the mutant ida background were conducted using the soybean (Glycine max) chitinase (CHIT) promoter (Broglio et al., 1989; Chen and Bleecker, 1995), a bean (Phaseolus vulgaris) abscission cellulase (BAC) promoter (Tucker et al., 1988), as well as the promoter of a new floral abscission marker, FLORAL ABSCISION ASSOCIATED GENE (FAA). Previous studies of CHIT::GUS and BAC::GUS in Arabidopsis indicated that expression of these genes was abscission zone-specific and associated with organ loss (Chen and Bleecker, 1995; Bleecker and Patterson, 1997). While FAA::GUS expression is not changed in the ida mutant background, this is the case for CHIT::GUS and BAC::GUS. This, in addition to in situ hybridizations of HAESA in ida provides us with new information on IDA’s role in floral abscission. Most notable, however, studies of plants with the IDA promoter driving GUS (IDA::GUS) in the mutant etr1-1 background show that the pathways regulated by IDA and by ethylene are interdependent.

**Materials and methods**

**Plant material and growth conditions**

Arabidopsis plants were cultivated in growth chambers at 20 °C for 8 h of dark and 16 h of light (100 μE m⁻² s⁻¹). Two ecotypes, Col and C24, were used.

CHIT::GUS (Chen and Bleecker, 1995) and BAC::GUS (Kochler et al., 1996) were introduced into the ida mutant background by crossing, with subsequent selection for the ida phenotype and GUS expression. A single-copy IDA::GUS line (Butenko et al., 2003) was crossed into the dominant etr1-1 mutant and the wild type Col. Plants were selected for the etr1-1 phenotype and GUS expression.

Segregation analysis showed that two independent T-DNAs were inserted into the genome of the BASIL GUS marker line (Stangeland et al., 2003). Genetic analysis and outcrossing identified the T-DNA insert that co-segregated with the GUS activity. The line with the single-locus T-DNA insert conferring the GUS expression to BASIL was crossed into the ida mutant and selection was done as described above.

**GUS assays**

GUS staining, and whole-mount clearing preparations of flowers, siliques, leaves, and pedicels from various positions along the inflorescence were performed as described (Grini et al., 2002) and inspected with a Zeiss Axioplan2 imaging microscope equipped with differential interference contrast optics and a cooled Axiscam camera imaging system.

**In situ hybridization**

For in situ hybridization, a HAESA PCR product described in Jinn et al. (2000) was cloned into pCR II-TOPO (Invitrogen, Carlsbad, CA). A 536 bp product from IDA spanning the entire exon in addition to 5’ and 3’ UTRs was cloned into pGEMT (Promega). Both clones were used as templates for digoxigenin-11-UTP labelling (Roche Molecular Biochemicals, Indianapolis, IN) of RNA probes.
HAESA, antisense RNA probes were synthesized with T7 RNA polymerase after linearization with SpeI, and sense controls were synthesized using Sp6 RNA polymerase after linearization with NotI. For IDA, antisense RNA probes were synthesized with T7 after linearization with SalI and the sense control was synthesized using Sp6 after linearization with NacI. In vitro transcription was performed using the manufacturer’s recommendations.

Flowers and siliques were fixed in 50% ethanol, 5% acetic acid, 3.7% formaldehyde, vacuum-infiltrated, and dehydrated in a graded ethanol series to 100%. The tissues were further stained with Eosin Y and passed through an ethanol:Histoclear series and embedded in Paraplast plus (Sigma).

Tissue sections (8 μm) were mounted on Superfrost® Plus slides (Fischer). Prehybridization, hybridization, and washes were performed as described previously (Jackson et al., 1994). The hybridized probe was detected by using the DIG Nucleic Acid Detection kit (Roche). Sectioned material was examined in the same way as GUS-stained whole mount specimens.

Wounding
The treatment involved mechanically wounding of about 50% of the area of the leaves with a pair of sterilized serrated forceps; about half the leaves of the rosette of a plant were wounded. Each pinch was made transversally across the leaf, leaving non-wounded material on either side of the wounded area. Wounding of the flower was done by mechanically injuring all floral organs by tearing part of the sepal, petals, and stamens off. On some flowers part of the siliques was also removed. Wounded and non-wounded plant material was stained for GUS activity 8 h post-wounding.

Results

Two abscission markers show reduced GUS expression levels in the mutant ida background

To track the abscission process in the wild type and in the mutant ida background, molecular markers for chitinase and cellulase were used. Developmental series of flowers were numbered corresponding to their positions on the inflorescence and stained for GUS activity. Position 1 corresponded to the flower at anthesis, when carpels, anthers, and petals are of approximately similar lengths. For both the CHIT::GUS reporter gene and BAC::GUS reporter gene, expression was found to be developmentally regulated in the AZs of sepal, petal, and filament (Figs 1A, 2A). In Col, CHIT::GUS expression (Fig. 1A) began to
The expression pattern of the BAC::GUS in wild-type (Fig. 2A) showed features similar to that of CHIT::GUS. However, the expression of BAC::GUS was detectable at earlier stages of development than CHIT::GUS as expression in the AZs of filaments, petals, and sepals began at anthesis and was clearly visible by position 1. In addition, the BAC::GUS expression was not only restricted to the AZ cells, like CHIT::GUS, but was also observed in the lower parts of the sepals, petals, and filaments. The highest level of BAC::GUS expression occurred prior to floral abscission at positions 4–6 and then began to decrease. As with CHIT::GUS, some GUS expression could be detected in sepal and filament abscission cells at later floral stages. In addition, BAC::GUS expression was also detected in the suture (AZ) of dehiscent anthers.

In the same manner as CHIT::GUS, the temporal expression of BAC::GUS in ida did not differ from the wild type, there was, however, a significant reduction in the AZ level of GUS expression (Fig. 2B). The GUS signal in the dehiscent anthers remained, however, unchanged in strength.

**BASIL is an abscission-specific marker acting independently of IDA**

An earlier screen of a collection of Arabidopsis promoter trap lines identified the GUS reporter line BASIL (base of the silique), where GUS activity was restricted to the floral AZs (Stangeland et al., 2003). An outcrossed BASIL line with a single T-DNA locus co-segregating with the GUS expression in the AZ (see supplementary material at JXB online), was investigated more closely. A strong GUS signal was detected in the AZ cells of the filament, petal, and sepal at position 6 in this line (Fig. 3A). The expression accumulated to maximal levels with the timing of floral abscission (position 8) and then slowly decreased, however, the GUS expression in BASIL remained longer than CHIT::GUS (Fig. 1A). In addition, a strong GUS expression was detected in the vestigial AZ of the pedicel temporally identical to the GUS expression in the floral AZ (see supplementary Fig. 1AI and III at JXB online). These results showed that BASIL could indeed be used as a marker to track the floral abscission process.

Cloning of the plant DNA flanking the right border of the T-DNA insertion using inverse PCR (Meza et al., 2002), revealed that the T-DNA was inserted on chromosome 4 in the gene At4g20010 that encodes a protein of unknown function (see supplementary Fig. 1B at JXB online). Arabidopsis plants transformed with a At4g20010 promoter–GUS construct displayed a GUS expression pattern equal to that seen in the BASIL line (see supplementary Fig. 1C at JXB online). At4g20010 has therefore been named the **FLORAL ABSCISSION ASSOCIATED GENE** (FAA).

The single-locus T-DNA line of BASIL was crossed into the mutant ida background to determine whether the GUS expression in BASIL would be altered. A developmental
series of flowers from this cross was examined for GUS expression. Contrary to CHIT::GUS and BAC::GUS the expression pattern in the ida background was indistinguishable from the wild-type expression both in AZ and at the base of the pedicel (Fig. 3B; see supplementary Fig. 1AII at JXB online).

In situ hybridization shows that HAESA expression is unaltered in ida

In order to determine the spatial and temporal expression pattern of HAESA in the mutant ida background, in situ hybridization was performed. In addition, in situ hybridization was done with an IDA probe to elucidate the endogenous pattern of expression for IDA further.

The RNA hybridization experiment with an antisense IDA probe demonstrated that IDA is expressed in the floral organ AZs at position 5 (Fig. 4AI), consistent with the reporter gene data (Fig. 5A). Unexpectedly however, IDA was also expressed in the dehiscence zone (DZ) of the silique, and this signal was present earlier in development, clearly detectable at position 3 (Fig. 4AI, II, III). The signal was retained as the silique matured (results not shown). HAESA was previously shown to be expressed in the floral organ AZs (Jinn et al., 2000). Here it is demonstrated that HAESA expression, like IDA, was not restricted to the floral AZ cells occurring with the onset of the floral abscission process, but was also expressed in the DZ of the developing silique (Fig. 4BI). In the mutant ida background the spatial and temporal expression pattern of HAESA was unaltered when compared with wild type (Fig. 4BII). All sense control probes gave no signal (Fig. 4C).

The spatial expression pattern of IDA is altered in the ethylene-insensitive mutant etr1-1

To determine whether ethylene could be influencing the regulation of IDA, IDA::GUS (Butenko et al., 2003) was crossed into etr1-1, which retains its floral parts to position 16 (Butenko et al., 2003). IDA::GUS expression is restricted to AZs of the corolla and filament anthers concurrent with the onset of the floral abscission process in C24 plants (Butenko et al., 2003). When a single-copy IDA::GUS plant was crossed into the Col wild-type background

Fig. 3. BASIL::GUS expression in wild type and ida. Cleared whole mount preparations of GUS-stained BASIL and ida flowers from different developmental stages. Stage-specific AZ GUS expression in (A) BASIL flowers and (B) mutant ida flowers. Top row, whole flower overview; middle row, AZs; bottom row, detailed view of AZ cells and separating sepal. Arabic numerals indicate flower positions on the inflorescence. Arrow heads indicate the three AZs at the junction between the filament (f), petal (p), sepal (s), and the floral receptacle. Notice the GUS expression in the separating cell layer on the organ side of the plant denoted by an arrow in (B).
the same pattern of GUS expression was observed (Fig. 5A). IDA::GUS was absent in flowers from positions 1 to 4. At position 5 a strong GUS signal was seen in the AZs of the floral organs. This specific signal was maintained throughout the floral abscission process. From position 8 the GUS signal could also be observed in the outgrowth of the nectaries and the vascular tissues leading away from the nectaries. As the siliques matured (position 13) this signal became restricted to the medial portion of the nectaries. During the course of abscission (from position 6 to 8), the GUS signal was found in the separating cell layers on the organ side and spread toward the petal, sepal, and filament apices in much the same way as BAC::GUS (Fig. 2A, B).

The GUS expression pattern in the etr1-1 plants crossed to IDA::GUS was temporally indistinguishable from that of IDA::GUS (Fig. 5A, B). Spatially, however, differences were seen as a distinct GUS signal was detected in the nectaries at position 5, but was not present in the AZs of the floral organs. The GUS expression in the nectaries remained strong during the developmental stages when abscission occurs in wild-type plants (Fig. 5A, B).

IDA is induced by wounding

To see if IDA would be up-regulated during wounding, GUS expression was looked at in a single copy line of IDA::GUS plants. IDA::GUS flowers at position 1 that were moderately wounded by removing half of the floral organs showed a distinct GUS signal in the floral AZ (Fig. 6II). Flowers that were severely wounded showed a strong GUS expression (Fig. 6III), while wounded rosette leaves from the same plant showed no GUS expression 8 h post-wounding. The results indicate that IDA is up-regulated in correlation with wounding, but only in cells where it is usually expressed.

Discussion

BASIL GUS expression is AZ-cell specific and can be used as a new molecular marker to track the floral abscission process

In the complex process of abscission, numerous gene products are likely to be involved at different stages, i.e.
differentiation of the AZ cells, sensitization of these cells to abscission signals, dissolution of the cell wall components, elongation of AZ cells, and the formation of a protective layer (Patterson, 2001). The identification of novel genes expressed during the abscission process will give a more complete understanding of the process, and provide new molecular markers. Here one such new marker is reported. The GUS activity in the promoter trap line BASIL (Stangeland et al., 2003) was very strong and strictly localized to AZ cells of filaments, sepals, and petals, in addition to the vestigial AZ of the pedicel (Fig. 3A; see supplementary Fig. AI at JXB online). Thus, the GUS expression was developmentally regulated by the floral and pedicel abscission process.

Fig. 5. IDA::GUS expression in wild-type Col and mutant etr1-1 flowers. Cleared whole mount preparations of GUS-stained IDA::GUS flowers from different developmental stages. Stage-specific AZ IDA::GUS expression in (A) Col wild-type flowers and (B) mutant etr1-1 flowers. Top row, whole flower overview; middle row, AZ; bottom row, detailed view of AZ cells and separating floral organs. Arabic numerals indicate flower positions on the inflorescence. The onset of the GUS expression in the filament (f), petal (p), and sepal (s) AZs in wild type are shown by solid arrow heads in (A), while the onset of the GUS expression in the nectaries of etr1-1 is shown with an arrow in (B). Notice the spreading of the GUS signal from the separating cell layers at the bottom of the separating floral organs towards the apices at positions 6 and 8 in wild type indicated by arrows in (A), and a similar pattern of expression for etr1-1 at position 8 although the signal is absent from the separating cells as indicated with an open arrow head in (B). Notice also the spreading of the GUS signal in the vascular tissue leading away from the nectaries in both wild-type and etr1-1.

Fig. 6. IDA is induced by wounding. Top panel: IDA::GUS flowers at position 1 and IDA::GUS rosette leaf. Bottom panel: wild-type flowers at position 1 and rosette leaf. (I) Uninjured flower. (II) Flower where half of the floral organs are removed. (III) Flowers where siliques and floral organs are injured. (IV) Wounded rosette leaf. Notice the increase in GUS expression (arrows) as more organs are injured (compare II to III).
pedicel separation (Cho and Cosgrove, 2000). In addition, ectopic expression of IDA leads to pedicel abscission (Stenvik et al., 2006), suggesting that numerous genes required for abscission are expressed in the vestigial pedicel AZ. It is therefore not surprising that FAA is expressed both in the floral AZ and the pedicel vestigial AZ; and indeed, this is also the case for HAESA (Jinn et al., 2000).

In the mutant \textit{ida} background there was no change in \textit{BASIL GUS} expression (Fig. 3B; see supplementary Fig. AII at JXB online), suggesting that this expression is not under the influence of \textit{IDA} and/or suggesting that \textit{BASIL} is acting upstream of \textit{IDA}. Interestingly, although the FAA expression pattern is associated with floral abscission, it is not dependent on the actual completion of cell separation. It would be relevant to investigate whether the \textit{BASIL GUS} expression pattern is altered in abscission mutants that are blocked earlier in the abscission process and in the line overexpressing IDA.

\textit{FAA} encodes a protein of unknown function (DUF731) belonging to a family with at least three other \textit{Arabidopsis} genes (At5g44785, At1g31010, and At1g47720), that all contain a RNA binding motif found in a protein that associates with the 5' UTR of the chloroplast \textit{psbA} mRNA in \textit{Chlamydomonas reinhardtii} (Barnes et al., 2004). A homozygous line with pDs-Lox inserted in the last exon of \textit{FAA}, WiscDsLox353B08 (Alonso et al., 2003), showed no aberrant abscission phenotype (data not shown) possibly due to redundancy of the At5g44785 gene which is also expressed in floral AZs (see supplementary Fig. 1E at JXB online). A double mutant between these two genes might give an indication of the role of these putative single-stranded binding proteins in the process of floral abscission.

Abscission markers show that IDA acts late in the floral abscission process

The markers \textit{CHIT::GUS} and \textit{BAC::GUS} have previously been used in \textit{Arabidopsis} to investigate where in the floral abscission process a mutant differs from the wild-type, to establish genetic pathways that regulate the floral abscission process (Bleecker and Patterson, 1997; Patterson and Bleecker, 2004). Analysis of \textit{dab} mutants containing the \textit{CHIT::GUS} and \textit{BAC::GUS} transgene demonstrated expression delayed in timing compared with the wild type, correlating with the delay in the abscission process of each mutant (Patterson and Bleecker, 2004). The situation is different in mutant \textit{ida} plants containing these reporter constructs, in that the GUS signal was clearly visible at the same time as in wild-type plants (Fig. 1, 2), thus placing IDA downstream of all the \textit{dab} mutants.

In wild type, onset of \textit{CHIT::GUS} expression (Fig. 1A) correlates with the initial loosening of the cell wall, and the \textit{GUS} expression peaks as the cells separate from each other. In \textit{ida} mutant plants with the \textit{CHIT::GUS} transgene, the same temporal and spatial pattern of expression is visible (Fig. 2B). However, the signal is significantly lower than in wild-type flowers, and never increases in intensity. These results indicate that the floral abscission machinery in \textit{ida} is initiated as in wild-type, allowing the expression of genes correlated with the initial stages of the abscission process. However, the culmination of the process of abscission is blocked in \textit{ida} plants, as is the expression of the abscission marker \textit{CHIT::GUS}. Again, this supports the idea that IDA acts during later stages of abscission. Since the shedding of plant organs provides an ideal site for invasion by pathogens it is not surprising that genes encoding pathogenesis-related (PR) proteins such as chitinase are up-regulated during the course of abscission (Lim et al., 1987; del Campillo and Lewis, 1992; Coupe et al., 1997; Wu et al., 2001). When the separation does not occur, as in the \textit{ida} mutant, it makes sense that a promoter of a gene involved in cellular defence, i.e. chitinase, is not up-regulated.

\textit{BAC::GUS} is turned on in the floral AZ at anthesis both in wild-type and \textit{ida} flowers, albeit no significant increase of GUS expression is seen in the \textit{ida} mutant (Fig. 2A, B). The \textit{BAC::GUS} GUS expression is not only restricted to the floral AZ but it is also present in dehiscent anthers releasing pollen. It is interesting to note that the significant reduction in the \textit{BAC::GUS} expression in \textit{ida} floral AZs is not observed in \textit{ida} anthers (Fig. 2A, B). Thus cell separation processes in other plant organs, for example, in anther dehiscence (Sander et al., 2001), are not affected in the \textit{ida} mutant. This observation is further supported by normal fertility of the mutant \textit{ida} (Butenko et al., 2003).

Cellulases have been shown to be involved in the disassembly of hemicellulosic cell wall components during organ abscission (Brummell et al., 1997). This observation that \textit{BAC::GUS} expression is turned on at the correct time in \textit{ida} argues that the mutant goes through an initial loosening of the cell wall. This and the \textit{CHIT::GUS} results, in addition to the drop in petal breakstrength and morphological development of the AZ fracture plane reported earlier (Butenko et al., 2003), further substantiate that IDA functions at the end of the abscission process, i.e. after the initial induction of both hydrolytic enzymes and PR-proteins, and downstream of the \textit{dab} genes.

\textit{IDA} and \textit{HAESA} have overlapping expression in both the floral AZ and the dehiscence zone of the silique

\textit{IDA} is predicted to encode a small secreted protein that potentially could function as a ligand (Butenko et al., 2003). As \textit{HAESA} is a plasma membrane-associated LRR-RLK involved in the control of floral organ abscission (Jinn et al., 2000), it was interesting to investigate the expression of \textit{HAESA} in the mutant \textit{ida} background (Fig. 4B). During the development of the flower, the \textit{HAESA} RNA signal was restricted to the AZ of the flower and DZ of the silique in
both wild-type and ida. Unlike the scenario for CLV3 and its putative receptor CLV1 where the CLV1 expression domain is enlarged in clv3 mutants (Fletcher et al., 1999), HAESA expression is not altered in ida. Both IDA and HAESA seem to be expressed in the same cells at the same time (Fig. 4A, B), distinct from CLV3 and CLV1 which are secreted and expressed from different cell layers (Fletcher et al., 1999). Thus the overlapping expression pattern does not necessarily indicate that HAESA is a receptor for IDA. Indeed there are four Arabidopsis genes that are closely related to HAESA, and one can therefore speculate that the antisense HAESA construct which resulted in a floral abscission phenotype (Jinn et al., 2000) reduced the level of expression of a closely related LRR-RLK which may very well be involved in an IDA-dependent signalling pathway.

The DZ expression of both HAESA and IDA (Fig. 4A, B), is interesting since the process of silique dehiscence, which aids seed dispersal, is accomplished through a co-ordinated programme of cell separation which share features in common with floral abscission (Rajani and Sundaresan, 2001; Dinneny and Yanofsky, 2005). Although lines overexpressing IDA show premature dehiscence of the siliques valves (Stenvik et al., 2006), the ida mutant shows no aberrant fruit dehiscence, and there have been no reports of HAESA involvement in silique separation. It is possible that both IDA and HAESA are functionally redundant, with other genes regulating the process of silique dehiscence. Indeed the myc/bHLH gene ALCATRAZ is expressed in various tissues, including the vestigial AZ of the pedicel, but is specifically involved in controlling cell separation in the DZ of the siliques (Rajani and Sundaresan, 2001).

Experiments where IDA::GUS plants were mechanically injured revealed another aspect of IDA expression; IDA is induced by wounding but only in tissues where it is normally expressed (Fig. 6). This premature induction of IDA in response to wounding might be part of programmed defence, as invasive stresses such as those resulting from wounding often lead to plants shedding the damaged organ (Taylor and Whitelaw, 2001). This supports the idea that genes involved in abscission can be turned on or off under specific physiological conditions that require the shedding of an organ and that there must be a cross-talk between the different pathways.

Ethylene-dependent and -independent abscission pathways may converge

Historically ethylene has been given a role as an important regulator of abscission (Brown, 1997). The availability of ethylene-insensitive mutants in Arabidopsis such as etr1-1 has, however, made it possible to dissect the role of this hormone in the abscission process (Bleecker and Patterson, 1997; Butenko et al., 2003; Patterson and Bleecker, 2004). In etr1-1 as well as the dab mutants, the reporter gene expression pattern for CHIT::GUS and BAC::GUS is delayed in timing corresponding to the delay in floral abscission, suggesting that the expression of these promoters is under the regulation of the abscission process itself, independent of whether or not the plant is capable of responding to ethylene (Bleecker and Patterson, 1997; Patterson and Bleecker, 2004).

HAESA::GUS shows indistinguishable GUS expression patterns in the wild type and the etr1-1 background (Jinn et al., 2000). Thus, HAESA acts completely independently of the ethylene signal transduction pathway (Jinn et al., 2000). Both ida seedlings and mature plants have normal ethylene sensitivity, but as the inhibition of floral abscission is unaffected by the exposure of exogenous ethylene (Butenko et al., 2003) IDA appears to be independent of ethylene responses. However, the spatial expression of IDA::GUS in etr1-1 differed from that in wild-type Col plants (Fig. 5A, B). In both Col and etr1-1 IDA::GUS is expressed in flowers from position 5 (Fig. 5A, B) concurrently with the initiation of cell wall loosening (Butenko et al., 2003). This demonstrates that IDA is turned on by a signal independent of ethylene. Nonetheless, whereas IDA::GUS is expressed in the floral AZ and floral organs, the expression is restricted only to the nectaries in etr1-1 (Fig. 5A, B).

These results support the idea that while ethylene may accelerate the process of abscission, genes like IDA are essential. Therefore, consistent with the fact that ethylene acts as a modulator of abscission-related pathways, IDA seems to be influenced by an ethylene-response pathway. Conversely, the delay in abscission in the etr1-1 mutant may, in part, be due to the restricted expression pattern of IDA, which is needed to complete the floral abscission process.

Our use of promoter–reporter gene markers of the abscission process has revealed several different expression patterns, as BASIL is abscission associated, but independent of the actual cell separation step, normal CHIT::GUS and BAC::GUS expression is by contrast dependent on cell separation, and IDA::GUS is modulated by ethylene in contrast to HAESA. It is proposed that several regulatory pathways may act on one single core pathway that actually drives the abscission process, and that the IDA and ETR1 may act in branching pathways that converge at some point to control the expression of genes that contribute to abscission. The involvement of ethylene in the regulation of IDA could be more pronounced during various stresses such as wounding which often leads to elevated levels of ethylene (Delessert et al., 2004). It will be important to identify additional members of these pathways using molecular and genetic tools, to distinguish how the different components regulating floral abscission in Arabidopsis interact. Our new abscission related markers and expression studies in different genetic backgrounds are contributions to unravelling this process.
Supplementary data

The supplementary material, which can be found at JXB online, provides a detailed description of the cloning of the plant DNA flanking the T-DNA in the BASIL line identifying the insertion in the FAA gene (At4g20010), and documentation of expression of FAA in floral and pedicel AZs. The supplementary information also provides colour versions of Figs 4, 5, and 6.

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