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Insight

Re-evaluation of the ethylene-dependent and -independent pathways in the regulation of organ abscission focusing on model plants of flower abscission

Shimon Meir[1*], Sonia Philosoph-Hadas[1], Joseph Riov[2], Mark L. Tucker[3], Sara E. Patterson[4], and Jeremy A. Roberts[5]

[1]Department of Postharvest Science of Fresh Produce, Agricultural Research Organization (ARO), The Volcani Center, Rishon LeZion 7505101, Israel

[2]The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel

[3]Soybean Genomics and Improvement Lab, Agricultural Research Service, United States Department of Agriculture, Bldg. 006, BARC-West, Beltsville, MD 20705, USA

[4]Department of Horticulture, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA

[5]Office of the Vice-Chancellor, 18 Portland Villas, Drake Circus, Plymouth, Devon, PL4 8AA, UK

E-mail address for each author:

Shimon Meir – shimonm@volcani.agri.gov.il

Sonia Philosoph-Hadas - vtsoniap@volcani.agri.gov.il

Joseph Riov - joseph.riov@mail.huji.ac.il

Mark L. Tucker - mark.tucker@ars.usda.gov

Sara E. Patterson - spatters@wisc.edu

Jeremy A. Roberts - jerry.roberts@plymouth.ac.uk

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*Corresponding author details:
Dr. Shimon Meir,
Dept. of Postharvest Science of Fresh Produce, Agricultural Research Organization (ARO), The Volcani Center, Rishon LeZion 7505101, Israel
Tel: 972-3-9683667
FAX: 972-3-9683878
shimonm@volcani.agri.gov.il

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Highlights: Ethylene is the key regulator of organ abscission, while the IDA-HAE-HSL2 pathway acts downstream of ethylene signaling. The involvement of the turgor pressure in the execution of abscission is suggested.

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ABSTRACT

Abscission is a developmental process with important implications for agricultural practices. Ethylene has long been considered as a key regulator of the abscission process. The existence of an ethylene-independent abscission pathway, controlled by the complex of INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptide and the HAESA (HAE) and HAESA-like2 (HSL2) kinases, has been proposed, based mainly on observations that organ abscission in ethylene-insensitive mutants was delayed but not inhibited. A recent review on plant organ abscission signaling **(Patharkar and Walker, 2018)**, highlighted the IDA-HAE-HSL2 components as the regulators of organ abscission, while the role of auxin and ethylene in this process was hardly addressed. After a careful analysis of the relevant abscission literature, we propose that the IDA-HAE-HSL2 pathway is essential for the final stages of organ abscission, while ethylene plays a major role in its initiation and progression. We discuss the view that the IDA-HAE-HSL2 pathway is ethylene-independent, and present recent evidence showing that ethylene activates the IDA-HAE-HSL2 complex. We conclude that the ability of an organ to abscise is tightly linked to cell turgidity in the abscission zone, and suggest that lack of cell turgidity might contribute to the failure of floral organ abscission in the *ida* mutants.

Key Words:

Abscission induction, abscission zone, Arabidopsis, desiccation, ethylene, flower organs, HAESA, HAESA-like2, IDA, turgor.

INTRODUCTION

Abscission is a natural developmental process, in which subtended plant organs, leaves, flowers, fruits, and branches, separate from the parent plant (Osborne, 1989; Lewis *et al.*, 2006). The abscission process has been described as having four stages (Patterson, 2001; Taylor and Whitelaw, 2001; Roberts *et al.*, 2002; Kim, 2014): **A,** Differentiation of undifferentiated cells to an anatomically discrete abscission zone (AZ); **B,** Acquisition of the competence of the AZ cells to respond to abscission signals; **C,** Activation of the AZ cells by the abscission signals, leading to cell wall loosening by newly synthesized cell wall hydrolyzing enzymes and rounding of the AZ cells, which result in organ separation (execution phase); **D,** Trans-differentiation of the retained portion of the AZ to produce a protective layer.

Ethylene was discovered as a gaseous plant hormone associated with organ abscission following its identification as the active component in illuminating gas, which caused leaf abscission in trees growing along urban streets (Abeles *et al.*, 1992; Bakshi *et al*., 2015; Chang, 2016). Hall (1952) suggested that auxin and ethylene together play a role in the timing of leaf abscission. Auxin has an important role in **Stage B** of abscission, as the basipetal polar transport of auxin from the distal organ towards the AZ renders it insensitive to ethylene, thereby delaying or preventing abscission. Auxin depletion is a process occurring in the initial stage of abscission, leading finally to organ abscission (reviewed by Meir *et al.*, 2015). Since the above mentioned early discoveries, a substantial body of evidence has been published supporting the role of ethylene as a key regulator of abscission (Jackson and Osborn, 1970; Addicott, 1982; Abeles *et al.*, 1992; Taylor and Whitelaw, 2001; Brown, 2006; Meir *et al.*, 2010). Flower organ abscission in Arabidopsis and other species starts following pollination, which is known to trigger a large number of developmental changes in flowers such as petal and anther senescence and abscission, operating through increased ethylene production in flower organs (O'Neill, 1997).

The knowledge of the role of ethylene in abscission is routinely utilized to control organ abscission in agricultural practices. For example, ethylene releasing compounds are used for thinning of flowers and fruitlets, while ethylene inhibitors are used for inhibiting abscission of flowers, leaves, fruitlets, and fruits (Cooper *et al.*, 1968; van Doorn and Stead, 1997; Michaeli *et al.*, 1999; van Doorn, 2002; Blankenship and Dole, 2003; Malik *et al.*, 2003; Beno-Moualem *et al.*, 2004; Ascough *et al.*, 2005; Roberts and Gonzáles-Carranza, 2007; Agusti *et al.*, 2008; Goldental-Cohen *et al.*, 2017).

The discovery that floral organ abscission in Arabidopsis was only delayed but not prevented in ethylene-insensitive mutants, led to the suggestion that developmental pathways independent of ethylene might contribute to floral organ abscission in this model plant (Bleecker and Patterson, 1997; Butenko *et al.*, 2003; Patterson and Bleecker, 2004). Organ abscission of flower petals, sepals, and stamens in the ethylene-insensitive Arabidopsis mutants occurred in flowers at post anthesis positions (P) 10-15, while in the wild type (WT) abscission occurred at P6-P8.

Two leucine-rich repeat receptor-like kinases (RLKs), HAESA (HAE) and HAESA-like2 (HSL2), and the INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptide ligand, were found to be essential for Arabidopsis floral organ abscission (Jinn *et al.*, 2000; Butenko *et al.*, 2003, 2006). Additionally, an Arabidopsis *ida* mutant in which the floral organs did not abscise throughout the development of the siliques was reported, and overexpression of *IDA* using the 35S promoter enhanced floral organ abscission (Stenvik *et al*., 2006). The *ida* plants are ethylene-sensitive as indicated by the observations that seedlings of the mutant showed the same triple response as WT seedlings, and mature *ida* plants exhibited enhanced leaf and flower senescence in response to ethylene (Butenko *et al.*, 2003). However, flower organ abscission did not occur in the ethylene-treated *ida* mutant. These observations led to the suggestion that the IDA-HAE-HSL2 complex contributes to an ethylene-independent abscission pathway (Jinn *et al.*, 2000; Butenko *et al.*, 2003; Butenko *et al.*, 2006). The involvement of orthologs of *IDA*, *HA*E, and *HSL2* in the abscission process has been demonstrated in other plant species (Tucker and Yang, 2012; Stø *et al*., 2015; Wilmowicz *et al*., 2018). Moreover, the *IDA* orthologs from citrus (*CitIDA3*) (Estornell *et al*., 2015) and litchi (*LcIDL1*) (Ying *et al*., 2016) were shown to be expressed during abscission, and their expression in Arabidopsis accelerated floral organ abscission. In addition, expressing the two *IDA* orthologs in *ida* mutants completely restored the abscission ability of the floral organs.

Genetic investigations demonstrated that once RLKs are activated by IDA, the RLKs relay a signal through a Mitogen-Activated Protein (MAP) kinase cascade, that ultimately activates KNOTTED1-LIKE HOMEOBOX (KNOX) transcription factors. These factors are suggested to induce the transcription of cell wall remodeling and degrading enzymes responsible for the cell separation process and the subsequent abscission of organs (Cho *et al.*, 2008; Niederhuth *et al.*, 2013). Recent reviews on organ abscission signaling in plants (Meng *et al*., 2016; Patharkar and Walker, 2018) further developed this model, focusing on the cell separation stage, and suggested

that the IDA-HAE-HSL2 are the signaling inducers of organ abscission. This model is supported by the ectopic abscission at branch points and pedicels, in which there are vestigial AZ expressing HAE and HSL2, resulting from overexpression of *IDA* (Butenko *et al*., 2006; Stenvik *et al*., 2006; Stø *et al*., 2015). The model focuses on factors downstream of IDA/ HAE/HSL, and does not address the role of ethylene, auxin, and other plant hormones in the abscission process.

In the present article, we review the related literature and present evidence that, although IDA is an essential abscission factor, the IDA signaling pathway acts in the last stages of the abscission process rather than in the initiation stage, and serves as a signal to coordinate organ separation and post-abscission events. We also suggest that the spatial expression of the *AtIDA* gene in the Arabidopsis AZ is regulated by ethylene, as ethylene was found to upregulate the *IDA* orthologs in several plant species in which these genes were examined (Stø *et al.*, 2015; Ying *et al*., 2016). These findings bring into question the hypothesis that the regulation of the IDA-HAE-HSL2 pathway is ethylene-independent, and suggest that the IDA-HAE-HSL2 pathway acts downstream of ethylene signaling. Finally, the genes coding for cell wall degrading enzymes were upregulated in the AZ of the *ida* and *hae hsl2* plant similar to their upregulation in the WT (Niederhuth *et al*., 2013). Based on these observations, we discuss the role of the IDA-HAE-HSL2 pathway in inducing the expression of genes coding for cell wall degrading enzymes necessary to fully complete the organ abscission process. We also discuss the importance of cell turgidity in the AZ cells to enable a normal abscission execution. Accordingly, we suggest that the inhibition of floral organ abscission in the *ida* mutants may result from the loss of cell turgor pressure due to desiccation, and not solely resulting from a reduced upregulation of few cell wall degrading or modifying enzymes as compared to the WT. Taken together, we reassert the role of ethylene as a key regulator of organ abscission, and integrate its role in relation to the IDA-HAE-HSL2 complex and other factors, into a modified abscission model that differs from the models that were recently published (Meng *et al.*, 2016; Patharkar and Walker, 2018).

The IDA-HAE-HSL2 complex does not initiate organ abscission in Arabidopsis

The decrease in petal breakstrength (a measure of the force required to pull a petal off the flower receptacle), which represents the process of petal abscission, is the result of increased cell wall degradation, which finally leads to separation of the petals from the flower. Arabidopsis flowers develop along a raceme, in which flower P1 is defined when petals are first visible at the

top of the inflorescence. In Arabidopsis WT, the flower petals fully abscise between P6 and P8, shortly after anthesis. In addition, there is evidence showing that the petal abscission process, expressed in terms of decreased petal breakstrength, starts in the WT prior to the detection of the upregulation of *IDA* gene expression in the AZ. Thus, a significant decrease in the petal breakstrength in the WT was reported to occur at flower P4 (Patterson and Bleecker, 2004; Butenko *et al*., 2003; Wei *et al*., 2010; Chen *et al*., 2011), whereas *pIDA::GUS* expression was first detected at P5 (Butenko *et al*., 2003, 2006; Cai and Lashbrook, 2008).

If IDA initiates the abscission process, it is expected that the inhibition of the decrease in breakstrength in the *ida* mutant would occur starting from the initial stage of abscission. However, in both the WT and *ida* mutant, the petal breakstrength decreased by about 85-90% at P10 relative to the initial breakstrength at P2 during flower development. Only beyond this position (P10) there was a phenotype in *ida* in which the breakstrength increased and the petal separation did not occur (Butenko *et al.*, 2003; Stenvik *et al.*, 2008, Shi *et al.*, 2011). Similarly, the petal breakstrength in the *hae hsl2* mutant decreased by about 65% of the initial breakstrength up to flower P10 (Cho *et al.*, 2008; Stenvik *et al.*, 2008; Shi *et al.*, 2011). Nonetheless, there was a delay in the decrease of petal breakstrength in the *ida* and *hae hsl2* mutants from flower P6 up to P10 (Butenko *et al*., 2003; Liu *et al*., 2013), which suggested that IDA and HAE/HSL2 are participating in the cell wall loosening process. These observations of breakstrength changes indicate that, similar to the WT, abscission in the mutants is initiated and leads to the activation of cell wall degradation at the early flower positions. The above data suggest, that even though the IDA-HAE-HSL2 complex has a role in the regulation of a subset of several genes involved in the actual cell separation stage, the inhibition of petal abscission in the *ida* and *hae hsl2* mutants cannot be fully explained by the inability of the IDA-HAE-HSL2 complex to coordinate the production or secretion of proteins that contribute to cell wall loosening.

Based on the above observations it can be concluded that the IDA-HAE-HSL2 complex is not involved in the actual induction of the organ abscission processes in the AZ, as recently suggested (Meng *et al.*, 2016; Patharkar and Walker, 2018), but it regulates later stages of the process.

A rapid desiccation of the petals may contribute to the inhibition of floral organ abscission in the *ida* mutants

The execution of **Stage C** of the abscission process in Arabidopsis is characterized by anatomical changes, including dissolution of the middle lamella and loosening of the cell wall. These changes coincide with elongation and increased volume of the AZ cells at the fracture plane, resulting in their rounded shape observed using scanning electron microscopy (Bleecker and Patterson, 1997; Patterson and Bleecker, 2004). It was previously shown that water movement from mature cells to growing cells is driven by the turgor pressure (Lockhart, 1965), which is essential for elongation and increased cell volume (Lockhart, 1965; Ray *et al.*, 1972; Braidwood *et al.*, 2014). Thus, the cell enlargement process cannot occur in desiccated flower organs or leaves. Scanning electron micrographs, taken during abscission of Arabidopsis flower petals, revealed that rounded cells could be observed in the AZ of the WT at the flower P7-P12 (Bleecker and Patterson, 1997; Butenko *et al.*, 2003). In contrast, a flattened fracture plane was detected in the AZ cells at similar flower positions (P8-P12) in the *ida* mutant, in which the flower petals, which never abscise, started to desiccate and wilt from flower P8 or P10 onwards (Butenko *et al.*, 2003; Cho *et al*., 2008; Gonzalez-Carranza *et al*., 2012). The presence of a flattened fracture plane in the AZ cells indicates a partial dissolution of the middle lamella and a lack of turgor pressure. In contrast to the desiccated flower petals of the Arabidopsis *ida* mutant that remain attached to the inflorescence, in the ethylene-insensitive *etr1-1* mutant, turgid and vivid floral organs were present until just before abscission, which finally occurred at flower P11-P15 in various Arabidopsis accessions (Bleecker and Patterson, 1997; Patterson, 2001; Butenko *et al.*, 2003; Patterson and Bleecker, 2004). Similar to the *etr1-1* mutant, the petals were turgid when they abscised in another ethylene-insensitive mutant, *ein2-1*, and also in the delayed abscission mutants (*dabs*). In addition, scanning electron micrographs of all these mutants showed a similar abscission morphology to that in the WT, namely the occurrence of rounded cells in the AZ (Patterson and Bleecker, 2004; Kim *et al.*, 2013). It is interesting to note, that early screenings performed to identify delayed abscission mutants in the Wisconsin T-DNA collection, revealed that these were actually mutants associated with stress and desiccation, which caused a delayed abscission phenotype (Patterson, personal communication). These observations indicate that the ability of an organ to abscise is also associated with the turgidity of the tissues and the formation of enlarged rounded cells in the AZ. Therefore, it is proposed that loss of turgor pressure in the AZ cells in the *ida* mutant may significantly contribute to the failure of petal abscission in this mutant.

IDA is most likely involved in a pathway controlling the final separation stages in the

abscission process. This could be a negative signaling pathway inhibiting a repair process induced by the initial dissolution of the middle lamella, or a positive signaling pathway governing the final separation. Interestingly, in plants overexpressing *IDA* in the floral organ AZ, separation occurred at the flower P4, and a significantly greater number of rounded cells was observed compared to the WT (Stenvik *et al.*, 2006). In the flower positions subsequent to P4, the AZ cells gradually enlarged, and from the flower P6 onwards the AZ was covered with a white substance. These observations further strengthen the relationship between IDA and AZ cell growth leading to the phenotype of enlarged rounded cells that are necessary for separation (Stenvik *et al.*, 2006), and further support our contention that IDA serves as a signal for post-abscission events.

Recently, it was shown that petal desiccation in the *ida-2* mutant started at P5, and wilted petals were observed at P8, with no detection of petal abscission, whereas in the WT (Col) the turgid petals abscised at P9 (Ying *et al*., 2016). In addition, further corroboration of the need for turgid cells during abscission was observed in transgenic Arabidopsis plants expressing *LcIDL1* isolated from litchi, in which the *LcIDL1* transcript was expressed with the native Arabidopsis *IDA* promoter in the *ida-2* mutant. In these transgenic plants, floral organ abscission was completely restored, and the fast desiccation observed in the *ida-2* mutant was prevented (Ying *et al*., 2016).

An additional example of the strong association between the turgor pressure and abscission of Arabidopsis flower petals is based on the results reported by Basu *et al.* (2013), who demonstrated that a functional IAA signaling pathway is necessary for abscission to occur. However, unexpected results were obtained when they used a trans-activation approach with the gain-of-function *AXR3-1* gene to suppress auxin responses specifically in the floral organ AZ cells. Floral organ abscission was inhibited in the *AXR3-1* plants, even though the AXR3-1 protein acts to repress auxin signaling by increasing the stability of the Aux/IAA proteins. These unexpected results could be explained by the observations that the *AXR3-1* plants exhibited visual signs of flower senescence, desiccation, and wilting at P8, the flower position in which abscission occurs in the WT. This result supports the hypothesis that desiccation of the AZ cells may inhibit cell separation.

Is the IDA-HAE-HSL2 pathway ethylene-independent?

It was originally proposed that the *IDA* gene controls an ethylene-independent pathway of floral organ abscission **(**Butenko *et al*., 2003). However, there is evidence that the expression of

the *IDA* gene in the AZ of Arabidopsis flower organs is promoted by ethylene, but ethylene may not be essential for the continuity of its expression during abscission (Butenko *et al.*, 2006). Thus, in *IDA::GUS* plants the onset of the expression in the filament, petal, and sepal AZ in the WT could be detected from the flower P5 and onwards, while in the *etr1-1* mutant the signal was not evident in the separating cells even at the flower P8 and P9. Furthermore, wounding of flowers at P1, in which *IDA* was not yet expressed, induced expression of the *IDA::GUS* in the flower petal AZ 8 h after wounding. Increased expression of this gene in the AZ was correlated with the severity of the organ injury (Butenko *et al.*, 2006). The possibility that the wounding effect on *IDA::GUS* expression was mediated by ethylene and other stresses has not yet been examined. It should be noted that a recent report (Sundaresan *et al*., 2015) showed that the ethylene production rate of Arabidopsis flowers was relatively high in the flower P2-P5, and it preceded floral organ abscission, which occurred at the flower P7. Additionally, in the *eto4* mutant, which overproduces ethylene, the ethylene production rate was twice as much as that of the WT, and as a result, the flower organs of this mutant abscised at an earlier flower position (P5). These results suggest that the induction of ethylene biosynthesis precedes the floral organ abscission and the up-regulation of the *IDA* gene, which occurs from P5 (Butenko *et al.*, 2006) in the Arabidopsis flower AZ.

Furthermore, ethylene induced the abscission-specific expression of soybean and tomato *IDA-like* genes, and treatment of soybean with the ethylene action inhibitor 2,5-norbornadiene, greatly delayed the increase in the *IDA-like* gene expression (Tucker and Yang, 2012). Ethylene also induced expression of the *IDA* gene in non-AZ tissues, such as petioles and leaf blades, in soybean (Tucker and Yang, 2012). In addition, a recent report demonstrated that ethylene induced the expression of *EgHSL* and *EgIDA* genes in the AZ of oil palm fruits (Stø *et al.*, 2015), and that ethephon induced the expression of the *IDA-like* genes in the AZ cells of the fruitlet peduncle in litchi (Ying *et al.*, 2016), as well as in the AZ of lupine flowers (Wilmowicz *et al*., 2018). In all the above studies, the increased expression of the *IDA* or *HSL* genes in the AZ was detected early, prior to the onset of abscission, indicating a direct effect of ethylene on *IDA* expression. These observations cast doubt on the hypothesis that the pathway initiated by IDA is solely ethylene-independent and suggest that the IDA-HAE-HSL2 pathway is promoted by ethylene, and probably controls the abscission processes downstream of ethylene. Recently, Ying *et al*. (2016) reached a similar conclusion and suggested that IDA acts downstream of ethylene in an ethylene-dependent abscission pathway. It seems therefore, that the hypothesis that the IDA-HAESA complex is an

ethylene-independent factor leading to floral abscission should be re-evaluated. Further experiments to examine the effect of *IDA* overexpression in the *etr1* mutant background are necessary for clarifying this issue**.**

Another example of the regulation of floral organ abscission in an ethylene-independent manner was also proposed by Wei *et al.* (2010). They reported that overexpression of the Arabidopsis DNA-binding One Finger (DOF) transcription factor (*AtDOF4.7*) yielded ethylene-sensitive plants. Since the *AtDOF4.7* lines did not exhibit accelerated floral organ abscission following ethylene treatment, the authors concluded that the *DOF* overexpression led to abscission failure in an ethylene-independent manner. However, the authors seemingly did not notice that the flowers at P1-P2 had already been wilted in the ethylene-treated *AtDOF4.7*-overexpressed plants compared to the WT flowers, which had turgid petals at P3 and P4 and abscised in response to the ethylene treatment (Wei *et al*., 2010). Indeed, a recent report of the same laboratory (Wang *et al*., 2016) demonstrated that ethylene regulates *AtDOF4.7*, which is involved in the *IDA*-mediated floral organ abscission pathway. Therefore, it is quite reasonable to suggest that the lack of abscission in the ethylene-treated *ida* or *DOF*-overexpressing plants is also due to a rapid desiccation of the flower organs rather than to ethylene-insensitivity.

The IDA-HAE-HSL2 pathway induces gene expression of the cell wall degrading enzymes in a very limited manner

The proposed model that the IDA-HAE-HSL2 complex is the inducer of organ abscission (Meng *et al.*, 2016; Patharkar and Walker, 2018) relies on the assumption that this complex induces the expression of genes that encode proteins that contribute to cell wall disassembly and ultimately lead to organ separation. However, based on microarray (Liu *et al.*, 2013) and RNAseq data (Niederhuth *et al*., 2013), IDA and HAE-HSL2 were reported to affect the expression of only a low number of Arabidopsis genes encoding cell wall modifying and defense-related enzymes involved in abscission. Liu *et al*. (2013) used a microarray analysis to identify genes that were downregulated in both the *hae hsl2* and *ida-2* mutants, including genes encoding cellulose (*CEL*) and xyloglucan endotransglycosylase/hydrolases (XTHs). Two *XTHs* genes and a *CEL5* gene related to cell wall remodeling, as well as genes related to cell wall loosening and expansion, were downregulated in both the *hae hsl2* and *ida-2* mutants at P4-P8 compared to the WT. All these genes belong to a cluster of genes that were upregulated between developmental stages 12 to 15a-15c (clusters 1 and 3 in Cai and Lashbrook, 2008). These genes were also upregulated between

these stages in the mutants, but to a lower level than in the WT. A comparative analysis of the transcriptomes of the Arabidopsis WT and the *hae hsl2* mutant at the developmental stage 15, in which abscission had already occurred in the WT, showed a differential expression of a surprisingly low number of cell wall hydrolase genes (Niederhuth *et al*. 2013), Thus, only 11 out of 189 annotated hydrolase genes were downregulated in the *hae hsl2* mutant compared to the WT, and no evidence was provided that any of these genes was specifically expressed in the AZ. Moreover, these 11 genes were actually upregulated in both the *hae hsl2* and *ida* mutants and the WT between stages 12-15 (Niederhuth *et al*., 2013), indicating that their upregulation in the mutants occurred without the involvement of the IDA-HAE-HSL2 complex, similar to the upregulation of 1772 other genes. The RNA-seq data in the supplementary files of this article were reformulated and presented by Kim *et al.* (2015). The analyzed data showed that most of the genes related to cell wall disassembly and formation of the protective layer were similarly expressed in the receptacles of the WT and the *hae-hsl2* mutant, and some of these genes were even upregulated in the *hae-hsl2* mutant compared to the WT (Kim *et al.*, 2015). The minor delay in the decrease of petal breakstrength obtained in the *ida* and *hae hsl2* compared to the WT (Liu *et al*., 2013), as well as the reformatted results by Kim *et al.* (2015), indicate that the final cell separation step controlled by the IDA-HAE-HSL2 pathway is effected by a low number of the cell wall hydrolyzing enzymes. Additionally, downregulation of the *pXTR6::GUS* and *pPGAZAT::GUS* expression patterns in the AZ of the *ida* and/or *hae hsl2* mutant inflorescences, including the 11 genes reported above, was also reported by others (Gonzalez-Carranza *et al*., 2012; Kumpf *et al*., 2013). The observations that a few genes were downregulated in the mutants may explain the slight delay in the decrease of the petal breakstrength in the *ida* and *hae hsl2* mutants from flower P6 up to flower P10 (Butenko *et al*., 2003; Cho *et al.*, 2008; Stenvik *et al.*, 2008; Shi *et al.*, 2011; Liu *et al*., 2013). However, it should be emphasized that the process of cell wall remodeling requires the involvement of many gene families related to cell wall degradation, defense, and development of a boundary layer that are specifically upregulated in the AZ. Numerous such genes were detected in various abscission systems including tomato flowers (Meir *et al.*, 2010; Kim *et al.*, 2015), fruits of apple (Zhu *et al.*, 2011), melon (Corbacho *et al.*, 2013) and olive (Gil-Amado and Gomez-Jimenez, 2013), fruitlets of litchi (Li *et al.*, 2015) and citrus (Xie *et al*., 2018), soybean leaves (Kim *et al.*, 2015), and lupine florets (Glazinska *et al.*, 2017). It seems therefore, that the IDA-HAE-HSL2 pathway appears to regulate only a subset of genes encoding cell wall modifying

proteins expressed in AZ cells.

Conclusions and perspectives

Based on our analysis of the literature, there is overwhelming evidence that ethylene plays a crucial role in the timing of Arabidopsis floral abscission. We believe that the recent model regarding the ethylene-independent pathway suggested to be governed by IDA-HAE-HSL2 requires further evaluation, particularly the mechanisms by which this signaling complex operates alongside ethylene in regulating organ shedding. The new models of abscission regulation have mostly been derived from genetic approaches based on ethylene-insensitive mutants, that showed delayed abscission phenotypes, and on the non-abscising phenotypes of *ida* and *hae hsl2* mutants. The fact that abscission in ethylene-insensitive mutants, like *etr1-1*, is delayed but not inhibited suggests, that in these mutants an alternative regulator of abscission can be activated at later stages of development, much after abscission occurrence in the WT. Nevertheless, in Arabidopsis WT plants and most other abscission systems, the increase in ethylene biosynthesis that coincides with auxin depletion, leading to increased sensitivity to ethylene, is the key regulator of the abscission process (Meir *et al.*, 2015). Therefore, further efforts should be devoted to explore the crosstalk between ethylene, IAA, and the IDA-HAE-HSL2 pathway in coordinating organ abscission. A summarizing scheme, which takes into account all the factors mentioned here, illustrates the sequence of events in the abscission process of Arabidopsis plants (Box 1).

Moreover, we suggest that a tight link between the turgor pressure and the 'rounding up' of the AZ cells may play a critical role in the final act of organ shedding. Accordingly, there is a need to reexamine the rate of desiccation of AZ tissues in both the WT and the mutant plants, such as the *ida* mutant, in which a flattened or broken fracture plane in the AZ cells is detected. One approach that might be of particular interest in this regard is to investigate how the IDA peptide affects the water relations of the AZ cells, and whether IDA has an effect on aquaporins in the AZ cells during the final stages of organ separation.

It seems therefore, that a visible simple process such as abscission of the floral organs of Arabidopsis is clearly a very complex process that involves multiple pathways, the exact interactions of which are yet to be determined, and other un-elucidated pathways. In addition, while the basic involvement of the IDA-HAE-HSL2 pathway in the Arabidopsis abscission process of floral organs is evident, there is still no data regarding the role of the *IDA-like* genes in other

plants from which they were isolated. For example, overexpression of the litchi *LcIDL1* gene caused earlier floral organ abscission in Arabidopsis (Ying *et al*., 2016), but the role of this gene in litchi is still unknown. Thus, the model of Arabidopsis floral organ abscission (Box 1) cannot be yet applied to other plant species.

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Figure legends

Figure 1. A simplified schematic model depicting the sequence of events in AZ formation and flower organ abscission in Arabidopsis. The abscission process is depicted in four stages. A, Differentiation of the abscission zone (AZ). B, Acquisition of the competence of the AZ cells to respond to ethylene. C, Activation of the AZ cells by ethylene and execution phase, synthesis of cell wall hydrolyzing enzymes, and water uptake by AZ cells to enable their growth and rounded phenotype, which lead to organ separation. D, Trans-differentiation of the retained portion of the AZ to produce a protective defense layer. We represent in the model the main stages of the abscission process, highlighting a differentiation between the roles of ethylene and the IDA-HAE-HSL2 pathway and the crosstalk (purple overlapping lines) between these two signals. This model delineates the role of ethylene in regulating the initiation and subsequent regulation of the abscission process following auxin depletion, and the regulation by the IDA-HAE-HSL2 signaling pathway at more advanced stages of abscission, when the breakstrength is already in the process of decreasing. This scheme is adapted and updated from Bleecker and Patterson (1997) and Kim *et al*. (2015).