

Distinct new players in processing peptide hormones during abscission

Mariana Schuster and Renier A.L. van der Hoorn*

The Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford,
Oxford, OX1 3RB, UK

*Correspondence: renier.vanderhoorn@plants.ox.ac.uk

Summary

Flower organ abscission in *Arabidopsis* is regulated by a peptide hormone that is released from its precursor by a network of redundant subtilases. An exciting new study describes how drought-induced flower abscission in tomato is regulated similarly, but distinct via a single, different subtilase that releases a very different peptide hormone.

Main Text

Abscission of leaves, flower organs, flowers and fruits is a timely matter. Abscission is regulated by both development and stress and has an enormous impact on the reproductive success of the species. In agriculture, timing of abscission determines yield. Tomato, for instance, can drop its entire flower or fruit via drought-induced abscission, a process that can have dramatic impact on yield. In an exciting new study published in *Science*, Reichardt *et al.* demonstrate that abscission of tomato fruits and flowers involves the release of a peptide hormone by a subtilase. This pathway has striking similarities with peptide signalling described for flower organ abscission in *Arabidopsis*, but the players are distinct and most interestingly, it involves a well-described peptide hormone, unknown to be a key player in abscission.

Pioneering work on the molecular understanding of organ abscission in plants was made by studying floral organ abscission in *Arabidopsis thaliana* [1]. In *Arabidopsis*, sepals, petals, and stamens abscise when pollination is complete. At this stage, the gene *INFLORESCENCE DEFICIENT IN ABSCISSION (IDA)* is expressed [2] in the abscission zone of the respective floral organs [3]. *IDA* encodes for a secreted precursor protein for a peptide hormone that activates the HAESA (HEA) and HAESA-LIKE2 (HSL2) receptor kinases [4, 5].

Intracellular signalling, mediated by a mitogen-activated protein (MAP) kinase cascade,

results in the expression of cell wall modifying enzymes required for cell-cell separation, such as polygalacturonases and pectinesterases [6].

Inspired by the pioneering IDA work in *Arabidopsis*, researchers set out to understand drought-induced organ drop in crop plants. The components of the ‘IDA pathway’ are conserved in most angiosperms [7] and cross-complementation experiments have shown that the IDA ortholog of citrus complements the *Arabidopsis ida* mutant [8]. Surprisingly, however, the new report by Reichardt *et al.* describes a similar pathway but with very different components that regulates drought-induced flower drop in tomato, expanding the known repertoire of regulators of this pathway [9].

IDA also exists in tomato and may be involved in developmentally regulated abscission, but its gene expression is low and does not respond to drought stress [9]. Reichardt *et al.* therefore adopted a different strategy to dissect the phenomenon at the molecular level. Harnessing the knowledge that subtilases regulate organ abscission, the authors hypothesised that abscission could involve a subtilase that releases a peptide hormone to activate the signalling. The authors identified tomato phytaspase-2 (SlPhyt2), a subtilase that is upregulated under drought stress and accumulates in the flower pedicel adjacent to the abscission zone. Transgenic tomato plants with depleted or overexpressed SlPhyt2 had reduced or increased drought-induced flower drop frequencies, confirming a role for SlPhyt2 in flower abscission.

To identify the corresponding peptide hormone, they next determined the substrate specificity of SlPhyt2 using Proteomic Identification of Cleavage Sites (PICS) [10] and then scanned all known precursors of peptide hormones in tomato for a putative SlPhyt2 cleavage site. They identified a family of eight genes encoding precursors of phytosulfokines (PSKs). PSKs are known peptide hormones, initially described to regulate plant growth [11] and later found to also regulate plant defences [12]. PSKs are perceived by the PSK receptor (PSKR), of which there are two in tomato [13]. Among the eight PSKs, only PSK1 and PSK6 are expressed in the abscission zone upon drought, making them the most likely candidates for this type of abscission. Using a synthetic PSK peptide that corresponds to the conserved domain of PSKs, Reichardt *et al.* demonstrated that SlPhyt2 is indeed required for PSK biogenesis and that mature PSK induces flower abscission, even in the absence of SlPhyt2 [9].

Thus, strikingly, the abscission systems of tomato and *Arabidopsis* are regulated similarly, but distinctly different. On the one hand, the abscission pathways are consistent in the nature

of their components: a subtilase that releases a peptide hormone that is recognised by a receptor kinase. On the other hand, the components of these pathways are distinctly different. A first surprise is that the peptide hormones and corresponding receptor kinases are unrelated. The involvement of PSKs in tomato flower abscission was not anticipated. A second surprise is that the cleavage site is distinctly different. Whilst proPSK cleavage follows an Asp residue (P1=Asp), proIDA cleavage involves a much broader sequence and seems insensitive to the residue at P1. A third surprise resides in the phylogenetically distinct subtilases cleaving PSK and IDA precursors. Whereas proPSK is cleaved by SlPhyt2, which represents the SBT1 subfamily [14], proIDA is processed by SBT4 and SBT5 family members. Furthermore, in contrast to the Arabidopsis signalling cascade, which contains multiple redundant subtilase acting on one precursor (IDA), the tomato cascade involves a single subtilase acting on several PSK precursors. The functional redundancy of subtilases in Arabidopsis has been a great challenge and was overcome by expressing EPI10, a pathogen-derived subtilase inhibitor [1].

Interestingly, phytoaspases were so far more associated with programmed cell death (PCD) in plants. Arabidopsis has a single phytoaspase (At4g10540) [15] but this family has 12 members in tomato, each having distinct expression patterns and PCD roles described for some of them [14]. Reichardt *et al.* now show that phytoaspases can also regulate processes other than PCD. Likewise, PSKs are conserved among plant lineages and have universal functions [12] but a role in abscission had not been described until now. Tomato has two PSK receptors, and PSKR1 is required for immune responses involving an intracellular calcium-based signalling cascade [13]. Given their role in immunity and defence, the roles of phytoaspases and PSKs in drought-induced abscission is therefore rather unexpected, raising questions on how these pathways evolved and are regulated to provide specificity.

Finally, since the two abscission signalling cascades both contain a subtilase releasing a peptide hormone: how can the identities of both the protease and the peptide hormone be so distinctly different? Has this resulted from recent convergent evolution co-opting different peptide hormones and their receptors, or is prohormone processing by subtilases ancient and has it diverged during plant evolution? Together these studies show the fascinating versatility of peptide signalling in plants, controlling different abscission types and other biological phenomena.

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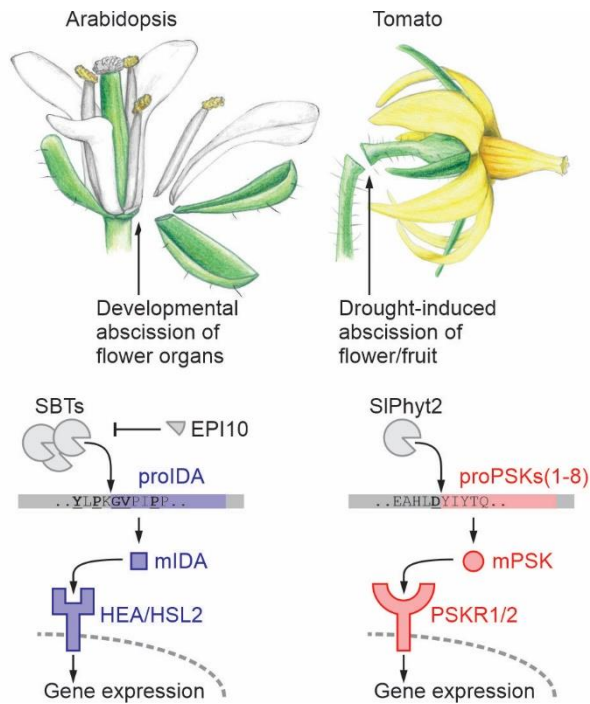


Figure 1 Distinct peptide hormone pathways regulating abscission of flower organs in Arabidopsis and tomato flowers/fruits, respectively. Abscission of flower organs (stamen, petals and sepals) in Arabidopsis occurs at the floral receptacles, whereas the abscission of flowers and fruits in tomato occurs at the pedicel. In Arabidopsis, the mature IDA peptide hormone (mIDA) is released from its precursor by a redundant group of subtilases that recognise the sequence YLPKGVPIIP in the IDA precursor (proIDA). mIDA is recognised by receptors HAE and HSL2, resulting in transcriptional reprogramming. In tomato, the mature PSK peptide hormone (mPSK) is released from their proPSK precursors (there are eight tomato precursors, all producing mPSK), by a subtilase (SIPhyt2) that cleaves after Asp residues (hence called phytaspase). mPSK is recognised by the corresponding receptors PSKR1 and -2, triggering transcriptional reprogramming.