Role of auxin depletion in abscission control

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Purpose of review: Abscission is a programmed developmental process initiated by auxin depletion. This review summarizes the mechanisms leading to auxin depletion in the abscission zone (AZ), evaluates the methods for estimation of the spatio-temporal auxin levels, demonstrates how auxin depletion occurs during natural, stress-induced, and artificially-induced organ abscission, and presents new evidence for early and late events resulting from auxin depletion which lead to organ abscission.

Findings: Auxin depletion occurs during natural developmental processes which end in organ abscission (leaf and flower senescence, fruit ripening, and self-pruning) and stress-induced abscission, and following artificial organ removal in the tomato model system. Stress-induced auxin depletion is mediated by increased ethylene and reactive oxygen species (ROS) production and carbohydrate starvation. Similar changes in auxin-related genes occurred in both flower AZ (FAZ) and leaf AZ (LAZ) following flower or leaf removal, respectively, suggesting a similar regulation of the abscission process of these organs. Auxin depletion resulted from decreased indole-3-acetic acid (IAA) biosynthesis and transport, as well as from enhanced IAA transport autoinhibition (ATA), conjugation and oxidative IAA catabolism. Functional analyses of several target genes delaying abscission, such as Knot-Like Homeobox Protein 1 (KD1), Tomato Proline Rich Protein (TPRP), Ethylene Responsive Factor 52 (ERF52), and Ribonuclease LX (LX), shed light on various events operating in response to auxin depletion in tomato FAZ and/or LAZ. The information gained allows a better understanding of the abscission process driven by auxin depletion, and might lead to development of improved methods for abscission control in horticultural crops.

Direction for future research: A better understanding of abscission regulation as it pertains to auxin depletion will require advanced molecular tools such as microarrays, new generation sequencing (NGS), transcriptomic, functional, and proteomic analyses of target genes and proteins found to operate in the abscission process.

Keywords: abscission zone; auxin homeostasis; carbohydrates; ethylene; functional analysis of target genes; IAA; ROS; tomato; transcriptome

Introduction

Abscission is a process in which vegetative and reproductive organs are detached from the mother plant at a specific layer in response to developmental, environmental, hormonal, and molecular cues [1-12*]. It is generally accepted that the basipetal polar flux of auxin from the distal organ towards the abscission zone (AZ) renders it insensitive to ethylene, thereby delaying or preventing abscission. The reduced auxin flow to the AZ as a result of decreased biosynthesis in the source tissue (the abscising organ), or inhibition of its polar auxin transport (PAT) are the main factors that render the AZ competent to respond to auxin signals [13-17**, 18, 19*, 20].

A widely accepted working model of abscission [7, 12*] defines four major stages in the abscission process: 1) differentiation of the AZ in the future site of organ detachment; 2) acquisition of competence of the AZ cells to react to auxin signals; 3) activation of the abscission process in the AZ and execution of organ detachment; 4) formation of a protective layer on the proximal surface of the separation layer.

It is well established that plant hormones apart from auxin and ethylene regulate abscission. Abscisic acid, jasmonates, and cytokinins act as abscission-accelerating signals [6, 8, 12*, 21-26], while gibberellins, polyamines, and brassinosteroids act as abscission inhibiting signals [6, 7, 12*, 27-31], but these effects will not be covered here. This review focuses on evidence that auxin depletion in the AZ cells is the main signal leading to acquisition of their competence to respond to ethylene by execution of organ abscission. The following issues will be addressed: 1) regulation of auxin levels in plants: biosynthesis, metabolism, transport, and signaling processes; 2) auxin depletion during natural and stress-induced abscission; 3) elucidation of the sequence of events leading to organ abscission following artificial auxin depletion in tomato (Solanum lycopersicum). New data, derived from recent customized tomato AZ-specific microarray experiments, show regulated changes in expression of auxin-related genes in the A/Zs following removal of their auxin source. These data reveal new auxin-related genes that were not associated until now with the abscission process.
Regulation of auxin levels in plants: biosynthesis, metabolism, transport, and signaling processes

The processes of auxin biosynthesis, metabolism, transport, and signaling have been extensively reviewed in recent years [32-35**, 36-40, 41*, 42*, 43-46**, 47, 48]. Tryptophan (Trp) is the main precursor for indole-3-acetic acid (IAA) biosynthesis in plants. There are several proposed IAA biosynthetic pathways [37, 42*, 48]. In Arabidopsis, the predominant pathway is the indole-3-pyruvic acid two-step linear IAA biosynthetic pathway, which is operated by Trp aminotransferase of Arabidopsis and flavin monooxygenase enzyme families encoded by the YUC-C4 gene family. Another two-step biosynthetic pathway that operates in bacteria and plants is the indole-3-acetamide (IAM) pathway. In this pathway, Trp is converted to IAM by Trp-2-monooxygenase (IAM), which is subsequently hydrolyzed to IAA by indole-3-acetamide hydrolase.

The distribution and homeostasis of IAA depend on both metabolism (biosynthesis, conjugation, and catabolism) and cellular transport. IAA conjugates play an important role as inactive storage forms of IAA [35**, 37]. In its free active form, IAA comprises only 5-25% of the total amount of IAA, depending on the tissue and plant species. The major forms of IAA conjugates are low molecular weight esters, such as IAA-glucose, synthesized by IAA-glucose synthase, and amide forms synthesized by the enzyme Gretchen Hagen3 (GH3). The IAA conjugates can be hydrolyzed to form free IAA by IAA-Leu resistant (ILR), IAA-Ala resistant, or ILR-like enzymes, whereas indole-3-acetyl aspartate and indole-3-acetyl glutamate act as precursors of a non-hydrolytic degradation pathway [37, 42*, 45, 48]. IAA catabolism has been shown to occur either by an oxidative decarboxylation pathway, leading to modifications of both the side chain and the indole ring, or by a non-decarboxylative oxidation of the indole moiety. Oxidative degradation of auxin appears to be developmentally important, mainly during fruit ripening and plant responses to oxidative stress [37]. Most auxin in biosynthetic and metabolic pathways occur in low rates, ranging between 10 nM/h to 1 µM/h, with the exception of auxin conjugation, which has rates as high as 100 µM/h [45]. Molecular and biochemical data discussed later in this review provide evidence that components of auxin homeostasis are regulated in the AZ tissues.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ARF</td>
<td>Auxin Response Factor</td>
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<td>ATA</td>
<td>IAA Transport Autoinhibition</td>
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<td>Aux/IAA</td>
<td>Auxin Resistant/Indole-3-Acetic Acid-Inducible</td>
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<td>AUX/LAX</td>
<td>Auxin Resistant/Like Aux</td>
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<td>AZ</td>
<td>Abscission Zone</td>
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<tr>
<td>DAP</td>
<td>Days After Pollination</td>
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<td>DPA</td>
<td>Days Post Anthesis</td>
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<td>ER</td>
<td>Endoplasmic Reticulum</td>
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<td>ERF</td>
<td>Ethylene Responsive Factor</td>
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<td>FAZ</td>
<td>Flower AZ</td>
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<tr>
<td>GH3</td>
<td>Gretchen Hagen3</td>
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<tr>
<td>GUS</td>
<td>β-Glucuronidase</td>
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<tr>
<td>IAA</td>
<td>Indole-3-Acetic Acid</td>
</tr>
<tr>
<td>iaaM</td>
<td>Tryptophan-2-Monoxygenase</td>
</tr>
<tr>
<td>ILR</td>
<td>IAA-Leu Resistant</td>
</tr>
<tr>
<td>KD1</td>
<td>Knotted-Like Homeobox Protein</td>
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<tr>
<td>LAX</td>
<td>Like Aux</td>
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<tr>
<td>LAZ</td>
<td>Leaf AZ</td>
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<tr>
<td>LX</td>
<td>Ribonuclease LX</td>
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<tr>
<td>1-MCP</td>
<td>1-Methycyclopentene</td>
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<tr>
<td>NAZ</td>
<td>Non-AZ</td>
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<tr>
<td>NGS</td>
<td>New Generation Sequencing</td>
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<tr>
<td>PAT</td>
<td>Polar Auxin Transport</td>
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<tr>
<td>PCD</td>
<td>Programmed Cell Death</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PG</td>
<td>Polygalacturonase</td>
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<tr>
<td>PILS</td>
<td>PIN-LIKES</td>
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<tr>
<td>PIN</td>
<td>Pin-formed</td>
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<tr>
<td>PM</td>
<td>Plasma Membrane</td>
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<td>qPCR</td>
<td>Quantitative PCR</td>
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<tr>
<td>REV</td>
<td>REVOLUTA</td>
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<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
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<tr>
<td>TAPG4</td>
<td>Tomato Abscission PG4</td>
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<tr>
<td>SAUR</td>
<td>Small Auxin Upregulated RNA</td>
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<tr>
<td>TF</td>
<td>Transcription Factor</td>
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<tr>
<td>TIRI/AFB</td>
<td>Transport Inhibitor Response1/Auxin Signaling F-Box</td>
</tr>
<tr>
<td>TPRP</td>
<td>Tomato Proline Rich Protein</td>
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<tr>
<td>Trp</td>
<td>L-Tryptophan</td>
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</table>

From the sites of biosynthesis, IAA is transported to other parts of the plant by diffusion or through active transport. The directional PAT system distributes auxin from the sites of biosynthesis to basipetal parts of the plant, and is mediated by influx and efflux carriers. The active influx of auxin in plant cells is mediated by the influx carriers, Auxin resistant 1/Like aux1 (AUX/LAX), while efflux carriers belong to the pin-formed (PIN) family of proteins [37, 38, 46**]. The PIN family includes eight members in Arabidopsis thaliana (AtPIN1–8), and ten members in both tomato and potato (SIPIN1-10; StPIN1-10, respectively) [49]. PINs are divided into “long” and “short” PINs according to the length of the hydrophobic protein domain [37, 46**, 49]. In contrast to the long PIN proteins, which are located in the plasma membrane (PM), the short AtPIN5 protein is localized in the endoplasmic reticulum (ER), and it participates in the compartmental localization and homeostasis of auxin. In recent years, a novel putative auxin transport facilitating family that also regulates intracellular auxin homeostasis in plants has been identified [41]. Named PIN-LIKES (PILS), this family includes seven members in A. thaliana [41*, 46**]. The PILS proteins contribute to auxin-dependent regulation of plant growth by determining the cellular sensitivity to auxin. PILS proteins regulate intracellular auxin accumulation in the ER, and thus auxin availability for nuclear auxin signaling [41*, 46**]. Data presented later in this review provide direct evidence for PIN and PILS signaling in tomato AZ tissues.

Auxin enhances its own efflux by rapid modulation of the abundance of PIN proteins. The transcription of PIN is under...
ATA is involved in apical dominance and abscission of domiZ

Two transcription factors (TFs) were found to regul ate auxin

TF genes are expressed in the auxin transport route s through

Auxin regulation of transcription events involves a  core pathZ

way consisting of the transport inhibitor response1 /Auxin sigZ

auxin resistant/IAAZinducible Z Auxin Response Factor (Aux/ 

lar and molecular mechanisms for canalization invol ve the

the canalization hypothesis proposed by Sachs [50].  The celluZ

the direct control of auxin, and PIN proteins are also postZ

transcriptionally regulated by auxin. The auxin feedback reguZ

lation of its transport direction and capacity is closely linked to

the canalization hypothesis proposed by Sachs [50]. The cellular and molecular mechanisms for canalization involve the auxin resistant/IAA-inducible - Auxin Response Factor (Aux/ IAA-ARF) signaling pathway [51]. A similar phenomenon of canalization is the high velocity IAA transport from a dominant organ, which inhibits the IAA export out of the dominated organ, thereby causing IAA transport autoinhibition (ATA) in the 'junction' of auxin transport from the two organs [52]. ATA is involved in apical dominance and abscission of dominated organs [52-55]. Other plant hormones (ethylene, cytoZ

kinins, gibberellins), small secretory peptides, and environmental signals (light, gravity, abiotic, and biotic stresses) appear to modulate auxin intracellular compartmentalization by affecting PIN expression and sub-cellular PIN trafficking [37, 46**]. Ethylene, cytokinins, jasmonates, and strigolactone modulate PAT via transcriptional and posttranscriptional regulatory mechanisms of auxin carrier transcription or trafZ

ficking. By regulating PIN1 levels in the PM, strigolactone can influence the capacity of bud-derived auxin to canalize towards the stem, and thus modulates bud activity and shoot architecture [54].

Two transcription factors (TFs) were found to regulate auxin biosynthesis, transport, and signaling: KANADI, which has a Myb-like domain, and REVOLUTA (REV), a Class III HomeZ

domain-Leucine Zipper TF [56, 57*, 58-62]. These two TFs have been shown to play antagonistic roles: KANADI acts as a transcriptional repressor and negatively regulates PIN1 expresZ

sion, while REV promotes PIN expression and function. Both TF genes are expressed in the auxin transport routes through the procambium, cambium, and phloem, thereby playing an important role in vascular tissue formation and canalization [60]. Microarray evidence for regulated REV expression in tomaZ

to AZs responding to auxin depletion is described below in this review.

Auxin regulation of transcription events involves a core pathZ

way consisting of the transport inhibitor response1/Auxin sigZ

naling F-box (TIR1/AFB) proteins, the Aux/IAA transcriptional repressors, and the ARF TFs. Perception of auxin stabilizes the co-receptor complex of TIR1/AFB and Aux/IAA proteins, which trigger the proteasome-dependent degradation of the Aux/IAA transcriptional regulators. Aux/IAAs regulate auxin-dependent gene transcription by forming dimers with ARF proteins. The auxin-dependent release of the ARF TFs leads to the onset of auxin-mediated transcriptional reprogramZ

ming [33, 37, 39, 43, 47, 48]. In Arabidopsis, six TIR1/AFB can interact with 23 different Aux/IAAs to form numerous coZ

receptor complexes. Each of the Aux/IAA may interact with up to 19 ARFs to regulate distinct sets of target genes that control different physiological processes. Genome-wide identification, functional analysis, and expression profiling of the Aux/ IAA gene family in tomato were previously reviewed [40]. Most of the Aux/IAA genes are rapidly induced by auxin, and the level of several Aux/IAA transcripts increases within a few minutes following auxin treatment. Therefore, the expression of these auxin-induced genes can be used as indicators for auxin activity in various tissues, including AZs [63, 64].

Auxin depletion during natural and stress-induced abscission

**Determination of IAA levels**

Direct measurements of endogenous levels of IAA (free and conjugated), using analytical [65, 66] or immunological [66] methods were developed. Few publications used this analytical method to demonstrate IAA depletion in the AZ during the initiation of the abscission process [67, 68, 69**, 70, 71**, 72]. Most determinations of auxin activity in the AZs were based on the expression of auxin-induced genes. Initially, few auxin-induced genes were cloned from the AZ, and their expression in the AZ was analyzed by polymerase chain reaction (PCR) [63, 64]. Later, expression profiles of auxin-induced multi-gene families in various abscission systems were analyzed using microarray [20, 37, 47, 57**, 67, 77**] [or NGS [78*, 79*] techniques. In transgenic plants, the synthetic auxin-responsive promoter element DR5 fused to β-glucuronidase (GUS), DR5:GUS, was used for evaluation of IAA levels in the AZs [70, 71**, 80, 81**]. The use of DR5:GUS has the advantage of visual assessment, but has a disadvantage for determining IAA depletion due to the high stability of the GUS protein as compared to the green fluorescent protein, which dissipates rapidly.

Therefore, the green fluorescent protein was used as a reporter system for studying the temporal expression profiles of promoters for estimation of IAA depletion [82]. In recent years a new auxin sensor, DII-VENUS, has been developed. This sensor, which is composed of domain II of the Aux/IAA fused to the VENUS fluorescent protein, provides high-resolution spatial-temporal information about auxin distribution and response [83]. Unlike the DR5 response, the DII-VENUS sensor is closely related to auxin concentrations, and does not depend on the levels of Aux/IAA and ARF proteins, thereby enabling its use as a quantitative tool. All of these methods monitor auxin concentration indirectly, via the expression of auxin-induced genes. In our opinion they act as in vivo bioassays, which have some weaknesses. Moreover, measuring auxin activity requires to consider the effects of inhibitors and repressors in the tissues and/or in the extracts. Hence, the analytical auxin measurements, based on gas chromatography-mass spectrometry determinZ

ation [65] are more accurate for the determination of low levels of IAA, and are therefore preferential for estimation of auxin depletion in the AZ tissues.

**Auxin depletion during natural abscission**

Abscission of leaves, flowers, floral parts, and overripe fruits is the last developmental stage of these organs, and is therefore regarded as natural abscission. The present review examines whether auxin depletion in these organs, which serves as auxin sources to their AZs, precedes the execution of ethylene-controlled abscission.

**IAA depletion during senescence-induced flower abscisZ

sion**

Flower senescence and abscission can be ethylene-dependent or independent [84, 85]. Since the basic premise of this review is that auxin depletion in the AZ increases the ethylene sensitivity of the AZ cells, we will focus on ethylene-dependent abscission of flower buds, open flowers, and petals. Endogenous IAA levels usually decrease during flower senescence in most flowers that subsequently abscise [86, 87]. Indeed, the abscission of
unfertilized or male flowers may be ascribed to the low levels of endogenous IAA, which is produced in the ovary [88]. In Begonia, abscising male flower buds contain only 1% of the IAA present in non-abscising female flowers, and the seasonal variation in male bud abscission coincided with reduction of the IAA content in the buds [89]. In Lilium, IAA levels decreased in the gynoecium and petals when the petals started to wilt and senesce, prior to their abscission [90]. IAA level and the expression of 50 auxin-related genes were analyzed in the outer tepals of two Lilium cultivars that differ in their abscission timing. Although both cultivars have fully formed AZs, Lilium longiflorum flowers wilted substantially during senescence prior to their late abscission, while those of the closely related Lilium longiflorum Asiatic hybrid (L.A.) abscised early without wilting [91**]. A clear correlation between auxin levels and abscission timing of both cultivars was found in relation to senescence markers. Thus, in L. longiflorum, both free and conjugated-IAA significantly increased as senescence progressed, while free IAA levels in Lilium L.A. remained low at all developmental stages from closed bud to abscission, and the portion of IAA-amide conjugate gradually increased. Consistent with the view that declining IAA levels precede abscission, the ARF7/19-like gene was up-regulated in the delayed-abscising genotype, while in the early-abscising genotype it was down-regulated [91**].

In Dendrobium cut flowers, the floral buds at the top of the inflorescence stalk exhibit early yellowing and abscise. Application of an auxin transport inhibitor or an auxin action inhibitor to the stigma of open flowers induced high flower abscission rates [92]. Removal of the open flowers at the distal end of the pedicel reduced the time to abscission of the remaining pedicel. IAA placed on the cut surface of the pedicel, counteracted the effect of flower removal. Application of different auxins delayed senescence and inhibited the abscission of open Dendrobium flowers [92, 93]. These results support the view that auxin is an endogenous regulator of abscission of Dendrobium floral buds and flowers. Several other reports show that exogenously applied auxins prevented or delayed abscission of flowers and floral parts, styles, and stamens [88]. Accordingly, auxin is applied to extend the vase life of several cut flowers, such as Geraldton wax flowers, Cestrum, and poinsettia [63, 88, 94, 95]. Genetic experiments with Arabidopsis mutants further demonstrated the role of auxin in petal abscission. Mutation in ARF2 [96] and ARF1 and ARF2 [97] delayed senescence and abscission of Arabidopsis petals. Flowers taken from mutants in which individual family members of the auxin influx carriers AUX1 and LIKE-AUX3 (LAX3) were down-regulated, exhibited early abscission. Manipulation of IAA levels in the AZ cells by activation of the bacterial IAA biosynthetic genes, IAA-L-lys-synthetase and iaaM, enhanced or delayed petal abscission, respectively [81**]. It was shown that IAA-Lys-synthetase reduced IAA levels in the cells by conjugating free IAA to IAA-Lysine, while iaaM promoted IAA levels by converting Trp to indole acetic acid.

**IAA depletion during senescence-induced leaf abscission**

Endogenous IAA decreases at the onset and during senescence of leaves, and auxin treatment can delay leaf senescence in some plants [98-100]. Most of the research on leaf senescence was done by using detached leaves in model systems which do not show abscission phenotypes (Arabidopsis or flag leaf in monocots). Therefore, the changes in IAA levels during abscission were not studied. Only one early report showed a positive correlation between the decrease in IAA content during senescence of Coleus leaves and their abscission [101]. Moreover, in monocots and some annual dicots there is no AZ at the base of the leaf, and the leaves senesce, wilt, and remain dry on the plant, a process termed marcescence. Therefore, the changes in IAA content during leaf senescence in these plants, including the Arabidopsis model system, are less relevant.

**IAA depletion during ripening-induced fruit abscission**

Fruits typically abscise at the overripe stage of development. In general, IAA levels decrease in fruit during ripening, and this decrease coincides with maturation of the seeds, in which most of the IAA is produced [80, 102-105]. The decrease in IAA levels starts in tomato fruit as the fruit reaches the breaker stage [80, 103-104]. The reduction of free IAA levels during ripening results from the decrease of auxin signal around the seeds [80, 102] and expression of the auxin transport gene families LAX and PIN with advanced ripening [80], as well as from increased conjugation of free IAA to its amide conjugates, as GH3 expression is sharply increased with ripening [103-104]. A similar decrease in IAA levels and an increase in GH3 expression and indole-3-acetyl-aspartate levels were also observed in grape berries after reaching the mature green stage [105, 106]. It is likely, therefore, that auxin depletion in fruit contributes to subsequent abscission competence. Unfortunately, most studies of auxin metabolism in ripening fruit were terminated at the ripe stage, without evaluating fruit at the overripe stage in which most actual abscission events occur. Only one exception was reported, in which IAA levels were monitored in oil palm fruit until abscission. Thus, in developing fruit, the content of IAA peaked between 60 and 100 days after pollination (DAP), and subsequently decreased to very low levels between 100 and 120 DAP just before abscission, which occurred 140 DAP [107**]. Auxin application delayed oil palm fruit abscission [108]. However, IAA depletion in fruit may be species-dependent, as in peach several reports demonstrated increased IAA level during fruit ripening, which is required for fruit softening [109-111].

There has been little work in which fruit ripening was correlated with events occurring in nearby AZs. Recently, two transcriptomic reports demonstrated changes in auxin-related gene expression in the AZs of mature olive and melon fruit [78*, 79*]. In olive, gene expression was studied 154 days post anthesis (DPA), when fruit started to ripen, and 217 DPA, when fruit abscised. Numerous auxin-related genes were down-regulated in the AZ 217 DPA, including auxin biosynthesis genes, ILRI, auxin transporters LAX1, LAX2 and PIN, TIR1, two AUX/IAA family members, three ARF family members, and several auxin-induced genes [78*]. In melon, gene expression was studied at three time points: 36, 38, and 40 DPA. The ethylene climacteric peak and abscission occurred 37 and 40 DPA, respectively. Between 36 to 38 DPA two genes encoding for auxin efflux carriers, four AUX/IAA genes, and one ARF gene were down-regulated in the AZ [79*]. Between 38 to 40 DPA, 13 other auxin-related genes were down-regulated. These reports provide the first direct evidence for auxin depletion in the AZ during mature fruit abscission.

Taken together, the reported data show that the processes of flower and leaf senescence and fruit ripening generally lead to
reduction of endogenous auxin levels, which in turn result in organ abscission. Sometimes a direct evidence for auxin depletion prior to organ abscission is lacking in these systems, as the experiments did not analyze IAA levels in the senesced organ or overripe fruit, but the general pattern was validated in various systems.

**IAA depletion during self-pruning of spring shoots in sweet orange (Citrus sinensis)**

Citrus shoot tips abscise at an anatomically distinct AZ that separates the top part of the shoots into basal and apical portions. This natural process, termed self-pruning, plays an important role in citrus floral bud initiation. Citrus microarray was used to monitor the expression of genes at several time points: 5 or 3 days before self-pruning, when the shoot tips started to fall, and at the beginning of self-pruning of spring shoots, when the AZ was activated [20]. Twenty-four auxin-related genes were differentially altered during these stages of self-pruning; genes encoding auxin-induced proteins were up-regulated, while genes encoding ARFs were down-regulated. The authors concluded that auxin depletion in the AZ of the spring shoots causes the AZ to become sensitive to ethylene and abscisic acid, which accelerate the abscission process of the shoot tips [20].

**Ethylene as a mediator of IAA depletion**

Ethylene is the main regulator of leaf and flower senescence and fruit ripening, and is proposed here as a modulator of auxin depletion in these processes. Unlike the synergistic interactions between ethylene and auxin controlling specific growth and developmental processes [112, 113, 114**, 115, 116], the control of both natural and stress-induced abscission (see below) involves antagonistic effects of ethylene and auxin. Several modes of action were suggested for this negative interaction. One of the regulatory effects of ethylene on auxin levels, operated through inhibition of auxin transport to the AZs, was demonstrated long ago [117-122]. Additionally, in various natural abscission systems, ethylene was reported to reduce auxin levels by increasing the rate of auxin conjugation [120-122].

**Auxin depletion during stress-induced abscission**

Abscission of different organs is a common response to various biotic, abiotic, and physiological stresses [2, 4, 6, 119, 123**]. Stress-induced abscission initiated by auxin depletion is mediated by three main intermediate modulators: ethylene, ROS, and carbohydrate starvation [119, 123**, 124**, 125]. Ethylene production increases in tissues subjected to many stresses, subsequently regulating their auxin levels [126-128]. Stress-induced ethylene may promote auxin depletion in various abscissing systems via inhibition of auxin transport to the AZs [117-122, 129, 130].

**ROS**

ROS can induce organ abscission [131-133] and application of antioxidants and ROS scavengers can inhibit abscission [67, 68, 134-137]. Several publications reviewed the interplay between ROS and auxin [124**, 138-140**]. ROS induced by stress conditions have an impact on auxin signaling by affecting auxin homeostasis at the levels of auxin biosynthesis, metabolism, and distribution. ROS stimulated auxin catabolism by increasing decarboxylative and non-decarboxylative oxidation of IAA, IAA conjugation (ester and amid forms), and *GH3* expression, and inhibited IAA transport by decreasing *PIN* expression [67, 68, 124**, 138, 139**, 140**]. ROS scavenging genes were induced by ethylene during abscission of citrus leaves, suggesting that ethylene induces ROS in this system [138].

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**Table 1: Examples of auxin depletion mechanisms operated in various stress-induced abscission systems.**

<table>
<thead>
<tr>
<th>Stress type</th>
<th>Plant and abscission system</th>
<th>Mechanisms for auxin depletion</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Domination</td>
<td>Apple fruitlets in clusters</td>
<td>Decrease in PAT; decrease in ATA</td>
<td>13, 16, 17, 143, 157</td>
</tr>
<tr>
<td>Carbohydrate starvation</td>
<td>Grapevine berries in clusters</td>
<td>Decrease in PAT; decrease in ATA</td>
<td>19*</td>
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<tr>
<td>(phloem-girdling)</td>
<td>Cowpea flowers and fruitlets</td>
<td>Decrease in PAT; decrease in ATA</td>
<td>14</td>
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<tr>
<td>High temperatures</td>
<td>Litchi fruitlets</td>
<td>Increase in IAA oxidation; Decrease in IAA level; Decrease in PAT</td>
<td>67</td>
</tr>
<tr>
<td>Chilling temperatures</td>
<td><em>I. sinensis</em> leaves</td>
<td>Increase in IAA oxidation; Decrease in IAA level</td>
<td>68</td>
</tr>
<tr>
<td>Chilling + high light</td>
<td><em>I. sinensis</em> leaves</td>
<td>Increase in IAA oxidation; Decrease in IAA level</td>
<td></td>
</tr>
<tr>
<td>Water stress</td>
<td>Cotton leaves</td>
<td>Decrease in PAT</td>
<td>158, 159</td>
</tr>
<tr>
<td></td>
<td>Cotton buds, flowers, and fruits</td>
<td>Decrease in IAA level; Increase in IAA conjugates level</td>
<td>160, 161</td>
</tr>
<tr>
<td></td>
<td>Citrus leaves</td>
<td>Decrease in expression of IAA signaling genes</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>Poplar leaves</td>
<td>Decreased expression of IAA signaling genes</td>
<td>163</td>
</tr>
<tr>
<td></td>
<td>Balsam fir needles</td>
<td>Decrease in IAA level</td>
<td>164</td>
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</tbody>
</table>
Carbohydrate starvation
Numerous stressors, like water stress, high and low light, high or chilling temperatures, and high sink organ domination, result in carbohydrate starvation. Abscission of premature fruit, leaves, flowers, and flower buds is related to low sugar content [13, 15-17, 141-146]. Auxin application can inhibit some stress-induced organ abscission [15, 146-148], suggesting that the shortage of carbohydrate is correlated with auxin depletion. In non-abscissing systems, sugars have also been shown to play pivotal roles as signaling molecules [149-155156*]. Thus, soluble sugars were reported to increase IAA biosynthesis, free IAA levels, and IAA transport in these systems [154-155], as well as to up-regulate early auxin biosynthesis and PIN1 genes, to promote PIN1 abundance in the PM and to down-regulate two genes of PAT inhibitors [156*]. We anticipate that sugar signaling may ultimately be found to accompany stress-induced decrease in carbohydrates that result in auxin depletion in source organs and their AZs. The mechanisms of auxin depletion in various stress-induced abscission systems are summarized in Table 1. Auxin depletion is expected to increase AZ sensitivity to stress-induced ethylene, thereby resulting in abscission.

Elucidation of the sequence of events leading to organ abscission following artificial auxin depletion in tomato
Tomato has been extensively used to study flower and fruit abscission processes. Tomato is a very convenient model system, since tomato plants develop a distinct AZ in the midpoint of the flower pedicel, referred to as a pedicel AZ or FAZ in different publications. The anatomy and development of the tomato FAZ is well established [165-167], as are the proteins involved in the regulation of the FAZ differentiation and development [168-173]. Since the eighties of the previous century, in-depth studies have defined enzymes involved in cell separation in various species. The AZ-specific polygalacturonases (PGs) and cellulases are particularly well characterized [174-184]. The AZ cells are defined as specialized cell types that differ from their adjacent cells in perception and response to ethylene and auxin [2, 4, 185-187]. Therefore, it is expected that specific TFs and genes will be specifically expressed in the AZ and not in the adjacent non-AZ (NAZ) cells. Indeed, in the first transcriptome microarray analysis of the tomato FAZ performed following auxin depletion, several genes were found to be specifically expressed in the FAZ and not in the basal portion (proximal) of the pedicel NAZ region [74**]. These genes include several TFs, such as TOMATO KNOTTED4, Homeobox-Leu zipper13, TOMATO AGAMOUS-LIKE2,12, TOMATO PROLINE RICH PROTEIN (TRP-PF1), KNOTTED1-LIKE HOMEODOMAIN PROTEIN1 (KD1), Phantastica, and Orate [74**]. In two transcriptome analyses comparing gene expression in the tomato FAZ versus the NAZ, including the basal portion (proximal) and apical portion (distal) pedicel regions, 89 and 1,255 genes, respectively, were found to be specifically expressed at anthesis in the FAZ cells, including genes encoding for TFs, hormone-related proteins, cell wall modification enzymes, lipid metabolism, and others [76**, 77]. Most interestingly, the AZ-specific gene set include TF genes that encode key regulators of meristem-associated functions, which may be regulated by a signaling pathway that requires auxin supplied from the flower before the onset of abscission. Suppression of one of the tomato shoot meristem-associated TF genes, tomato ETHYLENE-RESPONSIVE FACTOR52 (SlERF52) by RNAi, did not affect FAZ development, but significantly delayed pedicel abscission. This suggests that SlERF52 plays a pivotal role in transcriptional regulation in the FAZ [188**].

In an attempt to perform a functional analysis of 45 AZ-specific genes whose expression changed early in the tomato FAZ following auxin depletion, experiments based on virus-induced gene silencing in the FAZ and LAZ were conducted. Silencing nine of these 45 genes led to a significant retardation of pedicel and/or petiole abscission following abscission induction. The role of these nine genes was further examined by silencing each of them in plants stably transformed with antisense or RNAi constructs driven by an AZ-specific promoter, Tomato Abscission PGA (TAPG). The results showed that TAPG:antisense constructs of KD1 [71**] and of TRP-PF1 [189] strongly inhibited both pedicel and petiole abscission. Conversely, up-regulation of KD1 showed accelerated pedicel and petiole abscission [71**]. Complementary DNA microarray and quantitative PCR (qPCR) analyses indicated that regulation of abscission by KD1 was associated with a change in the abundance of genes related to auxin transporters and signaling components. Measurement of IAA content using the DR5:GUS auxin reporter assay, confirmed by analytical auxin determination, showed that change, s in KD1 expression modulated the auxin concentration and response gradient in the FAZ [71**].

In a transcriptomic analysis comparing gene expression in the tomato FAZ and NAZ, in the proximal and distal flanking pedicel regions at anthesis [76**], a region-specific expression of auxin-related genes was found: 1) METHYLESTERASE1, which converts IAA methyl ester to IAA, DWARF IN LIGHT1, which encodes an IAA-amido synthetase, and GH3.6 showed higher transcript levels in the FAZ than in the NAZ; 2) ARF9 was expressed at higher levels in the proximal (basal) than in the distal (apical) NAZ region; 3) GH3.1 and a Hooker1 homolog were expressed at higher levels in the distal than in the proximal NAZ region. This region-specific differential expression of genes involved in the determination of auxin levels suggests that a gradient of auxin concentration formed along the pedicel regions may be a key factor in regulating the timing of pedicel abscission [76**]. Indeed, by using the DR5:GUS reporter, such a gradient of IAA concentration was recently found in VF36 tomato plants, in which the FAZ was also divided into distal and proximal regions [71**]. Consistent with the initial location of the auxin-producing source tissue, this gradient occurred according to the following sequence: distal side of NAZ > distal side of FAZ > proximal side of FAZ > proximal side of NAZ [71**].

In order to elucidate the molecular changes occurring in the tomato artificial auxin depletion model system during acquisition of abscission competence in the FAZ following auxin depletion and during execution of pedicel abscission, our group performed a microarray analysis, using the Affymetrix 10K oligonucleotide Tomato GeneChip [74**]. In this system, the flower that serves as an auxin source is removed. Applying IAA after flower removal or inhibiting ethylene action using 1-methylcyclopropene (1-MCP) prior to flower removal inhibited pedicel abscission, suggesting that pedicel abscission results from auxin depletion and is ethylene-dependent [74**]. Based
Figure 1: Effect of leaf deblading and ethylene treatment (A) and flower removal (B) on the kinetics of petiole and pedicel abscission, respectively, in tomato explants. The debladed-leaf explants held in vials with water were prepared as previously described for the flower explants [72], and exposed to ethylene (10 ppm for 24 h). The percentage of accumulated pedicel or petiole abscission was monitored at various time intervals following organ removal. The results are means of four replicates (n=30 explants) ± SE.

Leaf deblading removes the natural source of auxin to an AZ and promotes abscission [64]. The only report so far describing the effects of leaf deblading on expression of auxin-related genes in the LAZ was performed in *Mirabilis jalapa* [64]. In this system, transcripts of two auxin-induced genes, *Mj-Aux/IAA* and *Mj-Aux/IAA2*, were down-regulated as a result of IAA depletion by leaf deblading or treatment with the IAA transport inhibitor, 1-naphthylphthalamic acid. Application of IAA to the cut end of the petioles inhibited their abscission and prevented the decline in the transcript levels in the LAZ [64].

To further define auxin-related members of tomato LAZ and FAZ transcriptomes, we recently refined an experimental system based on the abscission responses of debladed tomato leaves and removed flowers. Deblading of tomato leaves led to abscission of attached petals during a period of 8-12 days. Exposure of the debladed-leaf explants to ethylene treatment for 24 h accelerated petiole abscission, which was completed within three days after the ethylene treatment (Figure 1A), at a similar rate to that of flower pedicel abscission without ethylene treatment (Figure 1B). RNA collected during petiole and pedicel abscission allowed us to compare transcriptomic changes in the tomato LAZ and FAZ, respectively, through the use of a customized AZ-specific microarray chip. The chip included transcripts identified using NGS of RNA isolated from tomato AZs at various time points during organ abscission, as well as transcripts from the Solanaceae genomics network and National Center for Biotechnology Information databases [194]. Here we present for the first time results from these microarray analyses describing the changes in expression of auxin-related genes (Figures 2-6). Generally, there is a high similarity in the abscission process of tomato leaves and flowers, with a few exceptions. Most of the auxin-related genes are expressed in both AZs, but some members of different gene families are expressed specifically in the FAZ or the LAZ.

The results presented in Figure 2 show that most genes encoding auxin influx (*LAX* family) and auxin efflux (*PIN* family) carriers were rapidly down-regulated in both AZs following IAA depletion, thereby supporting the requirement for auxin for activation of its transport carriers. The few exceptions were related to genes that have very low expression levels, such as *LAX4,5* and *PIN2,8* in the FAZ and *PIN3* in the LAZ. The down-regulation of *PIN3* and *PIL5* family members in the two AZs provides the first indication that the regulation of intracellular auxin accumulation in the ER, expected to control auxin availability for auxin signaling in the nuclei of AZ cells, may be important for abscission regulation at the cellular level. Two *PIL5* were up-regulated in the FAZ and one in the LAZ following flower removal or leaf deblading, respectively (Figure 2), further supporting the role of this novel auxin carrier family in regulating auxin homeostasis [41]. The REV TF was quickly down-regulated in the FAZ and LAZ after abscission induction, suggesting that there is a regulatory mechanism for decreasing *PIN* expression and function in both AZs as a result of

on the transcriptomic results following the application of the abscission modulators, an abscission-inducing model was proposed in which the sequence of events occurring during tomato pedicel abscission was divided into two phases: early events (0 to 4 h after flower removal) and late events (8 to 14 h after flower removal). The early events probably lead to acquisition of ethylene sensitivity and abscission competence, while the late events include processes leading to the execution of pedicel abscission and development of the defense layer. According to this model, the decrease in IAA provides the first signal for abscission. Responses to auxin depletion included down-regulation of genes induced by auxin, such as *Aux/IAA* and other *TF* genes, and up-regulation of genes repressed by auxin. The late events included increased ethylene production, due to up-regulation of ethylene biosynthesis genes, such as the genes encoding 1-amino-cyclopropane-1-carboxylate synthase, which lead in turn to AZ-specific up-regulation of abscission-related genes. These genes include genes encoding cell wall-modifying proteins and pathogenesis-related proteins [74**], as well as genes related to the development of a protective layer on the surface of the remaining tissue [190]. The late events, which are ethylene-induced, were inhibited by 1-MCP pretreatment, while the early events were not affected by the inhibitor. On the other hand, IAA application immediately after flower removal inhibited all the cascade of abscission events and the changes in the expression of auxin-induced or auxin-repressed genes [74**]. Two later studies showed a rapid decrease of *Aux/IAA* [191] and *ARF* [70] genes after tomato flower removal. Additionally, quantitative and *DR5-GUS* data demonstrated a fast IAA depletion after flower removal [70]. These authors adjusted the proposed abscission model [74**] to the slower abscission rate obtained in their tomato system (0-8 h for the early events and 16-32 h for the late events), and included the various *ARFs* in the model [70].

Programmed cell death (PCD) was shown to be another late event involved in the abscission process. This was based on the data showing that inhibiting the activity of LX ribonuclease (LX), an enzyme associated with PCD, delayed tomato leaf abscission [192]. Indeed, hallmarks of PCD were identified in the tomato LAZ and FAZ during the late stage of abscission, and data showing that different abscission-related processes occurred asymmetrically between the FAZ proximal and distal regions were presented [193**]. This asymmetric distribution of various abscission-related processes might be related to the auxin gradient demonstrated recently in the tomato FAZ [71**].

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Figure 2: Microarray expression profiles of tomato auxin transport-related genes in the FAZ and LAZ at various time points following abscission induction. The assay included family members of the auxin influx carriers (AUX/LAX), auxin efflux carriers (PINs, PILS) genes, and an auxin-related TF gene (REV). Samples were taken from the FAZ at 0, 2, 4, 8, and 14 h after flower removal, and from the LAZ at 0, 24, 48, 72, and 96 h after leaf deblading and ethylene treatment as detailed in the legend of Figure 1. Gene expression levels are indicated with the colored code bars ranging from 0% (light blue) to 100% (dark blue). Expression levels are based on the percentage of change of the average intensity values of the replicated samples for each time point. The numbers indicated in the first box of each sample represent average intensities values for all replicates at 0 h. Corresponding *Solanum lycopersicum* (Solyce) ID and gene names are presented in the left and right sides, respectively.

Figure 3: Microarray expression profiles of tomato IAA-related genes associated with IAA conjugation in the FAZ and LAZ at various time points following abscission induction. The assay included members of the GH3 and ILR gene families. All other details are as described in the legend of Figure 2.
Figure 4: Microarray expression profiles of tomato auxin-signaling genes in the FAZ and LAZ at various time points following abscission induction. The assay included members of the Aux/IAA gene family. All other details are as described in the legend of Figure 2.

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Figure 5: Microarray expression profiles of tomato auxin-signaling genes in the FAZ and LAZ at various time points following abscission induction. The assay included members of the ARF gene family. All other details are as described in the legend of Figure 2.

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auxin depletion. Interestingly, it was recently reported that over-expression of tomato *SlREV* TF caused the transgenic plants to produce ectopic flowers from the pedicel AZ [195].

The highly expressed *GH3* genes significantly decreased after leaf deblading (Figure 3), further confirming that *GH3* is an auxin-induced gene [33, 35**, 37]. On the other hand, most *GH3* genes in the FAZ were up-regulated, confirming that increased IAA conjugation is involved in the process of auxin depletion [104, 105]. To the best of our knowledge, there are no reports regarding changes in *GH3* gene expression in the AZs after auxin depletion. In contrast to our earlier report describing up-regulation of *ILR* genes in the FAZ and the proximal NAZ [74**], in the current experiment the *ILR* genes were down-regulated in both the FAZ and LAZ after removing the auxin sources (Figure 3). Further investigation is required to understand the significance of these changes.

All the *Aux/IAA* genes that were expressed in the FAZ were also expressed in the LAZ (Figure 4). *IAA14*, *IAA9*, *IAA6*, and *IAA26* were the most highly expressed genes in both AZs. All IAA-related genes were down-regulated in both AZs within 2 h following abscission induction, except for *IAA6*, *IAA14*, and *IAA26*, whose expression in the FAZ was reduced after 8 h. These results are in agreement with previous reports regarding the timing of changes in the expression of IAA-responsive genes in the FAZ following flower removal [74**, 191].

Six *ARF* genes were exclusively expressed in the FAZ and three in the LAZ, and all *ARF* genes were down-regulated after IAA depletion in both the FAZ and LAZ (Figure 5). These results cannot be compared to previously reported results for *ARF* genes in the tomato FAZ [70], because in the previous system ethylene was applied after explant preparation. Out of 38 *Small Auxin Upregulated RNA (SAUR)* genes, 19 genes were exclusively expressed in the FAZ and four in the LAZ, and a high variability in the changes of the expression pattern of *SAUR* genes was found (Figure 6). There are no previous reports on the expression of *SAUR* genes in the AZ.

The present research compared for the first time the FAZ with the LAZ, showing similar changes in auxin-related genes in both AZs. Additionally, the results show that *Aux/IAA* genes are the best markers for determining auxin depletion in the AZs, and that the polar and intracellular auxin transport mechanisms are impaired after auxin depletion. Several IAA-related genes were shown for the first time to be involved in the abscission process, including *PIL5*, *PIN5*, *REV*, and *SAUR*.
genes. Taken together, the new data expand the range of various IAA-related genes that are rapidly down-regulated in the tomato AZs following auxin depletion as a result of flower removal or leaf deblading. Several recent articles further expanded the previously suggested model [7-9] for the sequence of events leading to pedicel abscission following artificial auxin depletion in tomato flowers. The new data were based on functional analyses of few target genes including KDT1, TPRPR, SELLFS2, and LX, whose silencing by virus-induced gene silencing, antisense or RNAi delayed abscission. Thus, the early events following auxin depletion include down-regulation of IAA-related genes encoding PAT components, auxin carriers, KDT1, and TPRPR, while the late events include up-regulation of ethylene-related genes, such as SELLFS2. These lead in turn to activation of cell wall hydrolyzing enzymes and PCD processes, which coincided with the increased expression of genes encoding cell wall degrading enzymes and up-regulation of LX, respectively. All these events finally result in the execution of pedicel or petiole abscission and formation of a protective defense layer on the remaining tissue.

Conclusions

This review provides evidence that auxin depletion in the AZs is required for AZ cells to react to ethylene as an abscission signal. This is a general phenomenon that occurs in natural abscission, in abscission induced by various stresses, and in the artificial system of tomato organ abscission after removal of the auxin source. The stress-induced auxin depletion is mediated by three main intermediate modulators, ethylene, ROS, and carbohydrate starvation. Auxin depletion can result from decreased IAA biosynthesis and transport, accelerated ATA, and increased oxidative IAA catabolism and conjugation. More target genes for functional analysis were elucidated by transcriptomic studies, using NGS or microarray in different abscission systems. The artificial auxin depletion system in tomato was found to be a very useful system for elucidating the sequence of events leading to organ abscission.

Direction for future research

In this stage of abscission research, with multiple target genes that might affect the abscission process, five future research directions are necessary: 1) performing transcriptomic analyses with more time points following abscission induction by auxin depletion, similar to those performed for the tomato FAZ; 2) analyzing the full transcriptome of the tomato LAZ for elucidating the sequence of events as in the tomato FAZ; 3) continuing the functional analyses of more target genes found in various abscission systems; 4) evaluating the importance of PIF3 and PIFS genes family members for auxin signaling in the nucleus of the AZ cells for abscission regulation at the cellular level; 5) establishing a proteomic system to elucidate the changes in proteins occurring in the AZs following auxin depletion, and examine if these changes are related to the changes of their gene expression.

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Papers of interest have been highlighted as:
* Marginal importance
** Essential reading

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23. Stips D and Eirnser J. Cytokinin stimulation of abscission in lemon pistil extract


36 **An updated review on auxin transport.**


48 An important article describing the role of REVOLUTA protein in auxin transport and signaling.


50 **An updated review showing the recent findings in auxin biosynthesis.**


59 **An important article elucidating the function of KD1 in auxin transport during flower and leaf abscission. The article also includes a visible demonstration of the
auxin gradient in the flower pedicel and the flower AZ.


** An intensive study showing changes in gene expression in the AZ and NAZ during various time-points following auxin depletion by flower removal, and in response to I-MCP and IAA application. This study served as the basis for the suggested detailed sequence of events operating from abscission initiation until the abscission execution.


** This study reveals at itsthesis a region-specific gene expression in the flower AZ and NAZ (proximal and distal regions to AZ).


* This study describes a transcriptomic analysis in the AZ during mature olive fruit abscission.


* A description of a transcriptomic analysis in the AZ during mature melon fruit.


** A very important article demonstrating the effects of genetic manipulation of auxin levels on petal abscission.


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** An excellent updated review describing ROS effects on auxin depilation.


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