

REVIEW

Recent advances in molecular events of fruit abscission

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Abstract

It is widely accepted that fruit abscission is a highly regulated developmental process that is both influenced and activated in response to changing environment and plays crucial roles in the health and reproductive success of plants. Recent evidences showed that numerous genes related to metabolic and signalling pathways were coordinately implicated in regulating fruit abscission. Cross talks within hormones, between saccharides and hormones, as well as between polyamines and ethylene result in synergetic or antagonistic interactions which together play an important role in adjusting fruit abscission. Although hormones are the most studied internal factors related to abscission, the role of saccharides and polyamines during fruit abscission is emerging now. The characterizations of the molecular mechanisms of regulating fruit abscission are essential to develop effective strategies for controlling this process in plants.

Additional key words: abscisic acid, cross talk, ethylene, gibberellic acid, indole acetic acid, jasmonic acid, polyamines, saccharides.

Introduction

As sessile organisms, plants have evolved a sophisticated fruit abscission mechanism in order to propagate successfully and respond to pathogen attack, drought stress, chilling injury, as well as hormone and nutrient imbalance (Li *et al.* 2010). Fruit abscission is a highly coordinated process that is finely mediated by phytohormones, saccharides, polyamines (PAs), H₂O₂, *etc.* All these compounds derived from plant biosynthetic or catabolic pathways can function either at the site of

generation or elsewhere through their transport in the plant (Iglesias *et al.* 2006, Dal Cin *et al.* 2009a, Gil-Amado *et al.* 2011, Ish-Shalom *et al.* 2011). However, these compounds do not act independently but are interrelated by synergetic or antagonistic cross-talk resulting in mediating biosynthesis or response which subsequently up- or down-regulates the expression of a set of genes related to fruit abscission. Based on the above-mentioned, it has been suggested that an extremely

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Abbreviations: ABA - abscisic acid; ACC - 1-aminocyclopropane-1-carboxylic acid; ACO - 1-aminocyclopropane-1-carboxylic acid oxidase; ACS - 1-aminocyclopropane-1-carboxylic acid synthase; ADC - arginine decarboxylase; ADE - adenylyate; AFLP - amplified fragment length polymorphism; AVG - aminoethoxyvinylglycine; AZ - abscission zone; CMNP - 5-chloro-3-methyl-4-nitro-1H-pyrazole; DAO - diamine oxidase; D-SAM - decarboxylated S-adenosyl methionine; EG - β -1,4-glucanase; GA - gibberellin; HKX - hexokinase; IAA - indole acetic acid; JA - jasmonate; LOX - lipoxygenase; LTP - lipid transfer protein; MCP - 1-methylcyclopropane; MTA - 5'-methylthioribose; NAA - naphthaleneacetic acid; NO - nitric oxide; ODC - ornithine decarboxylase; PA - polyamine; PAO - polyamine oxidase; PAT - polar auxin transport; PG - polygalacturonase; PLA₂ - phospholipase A₂; Put - putrescine; ROS - reactive oxygen species; SAM - S-adenosyl methionine; SAMDC - S-adenosyl methionine decarboxylase; S6PDH - D-sorbitol-6-phosphate dehydrogenase; Spd - spermidine; SPDS - spermidine synthase; Spm - spermine; TFs - transcription factors; TPS - trehalose-6-phosphate.

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complex signal transduction system is involved in abscission, and likely novel mechanisms remain to be discovered.

In recent years, significant research progress has been made in understanding signal transduction pathways (Mahouachi *et al.* 2009, Zhu *et al.* 2011). Studies using genetic screening and biochemical analytical methods have identified some important fruit abscission-related signal receptors and downstream signal components as well as cell wall degradation genes which are helpful in advancing our knowledge of fruit abscission mechanism. However, because of the limited availability of abscission related genes and their corresponding functions, understanding the molecular events associated with fruit abscission remained elusive.

The differentiation of abscission-zone cells

In order to escape from adverse environmental conditions and be successfully reproductive, plants have developed a fine-tuning abscission mechanism during the long period of evolution. For instance, under serious drought stress, abscission of leaves, flowers, and fruits can minimize the transpiration rate and it is important for the plant survival. To guarantee reproductive success, plants remove redundant or less-productive fruitlets in order to provide the remaining ones with enough nutrients for producing more viable seeds. The mature fruits have to be abscised in order to facilitate seed dispersal.

An essential requirement for an understanding of fruit abscission is knowledge of the structure of abscission zone (AZ). Previous anatomical observations of AZ indicated that it consists of several bands of small cells characterized by square shape and containing a dense cytoplasm (Sexton and Roberts 1982) (Fig. 1). Their differentiation may initiate very early or relatively late in the development of fruit (Sun *et al.* 2009) and be regulated by numerous different transcription factors (TFs). By analyses of AZ mutants, four of the main genes involved in AZ formation, *JOINTLESS*, *MACROCALYX*, *LS*, and *BLADE-ON-PETIOLE (BOP)*, have been identified. Using map-based cloning techniques, the *JOINTLESS* gene has been identified as a MADS-box gene that plays a pivotal role in controlling only the AZ development of the pedicel but not of the leaf, the style, and the corolla (Mao *et al.* 2000), showing tissue specificity. Most recently, Nakano *et al.* (2012) showed that physical interaction between the *JOINTLESS* and *MACROCALYX* protein, forming a heterodimer, regulates this process through phytohormone-related functions, cell wall modifications, fatty acid metabolism, and transcription factor activity. The promoter activities of this gene can be strongly induced by shading and exogenous naphthaleneacetic acid (NAA) (Zhu *et al.* 2011), indicating that the *JOINTLESS* gene is a hormone- and radiation-responsive gene and at the same time

Several years ago, reviews on abscission-associated signalling and its biological background have been published (Taylor *et al.* 2001, Racskó *et al.* 2006) showing that the molecular events of fruit abscission are obviously different from those of leaf or flower abscission even at different stages in fruit development. In light of fruit abscission as one of the most frequent problems leading to a serious economic loss in practice, we focused this review to highlight the role of hormones, saccharides, PAs, and their cross-talks in regulating fruit abscission and also on the molecular events implicated into fruit abscission. Our aims are to provide some useful information for future research in this field and to develop effective strategies for controlling the process in plants aiming at the improvement of fruit-tree cultivation.

providing evidence that at least an irradiance or a hormone or both signals are implicated in controlling AZ formation. This hypothesis is further supported by the fact that cytokinins slightly determined the formation of a secondary AZ in pear explants (Pierik *et al.* 1980). Regarding the *LS* gene (a tomato mutant lateral suppressor), it encodes a new member of the VHIID protein family that is also considered as transcription activator (Schumacher *et al.* 1999) which controls the formation of the pedicel AZ. Overexpression of the *NtBOP2* gene in tobacco plants leads to a failure in corolla abscission due to the formation of abnormally elongated AZ cells caused by perturbation of *NtBOP2* function. Furthermore, *NtBOP2* controls AZ development through interaction with the TGA transcription factor (Wu *et al.* 2012). These data support the idea that AZ formation may be initiated simultaneously with

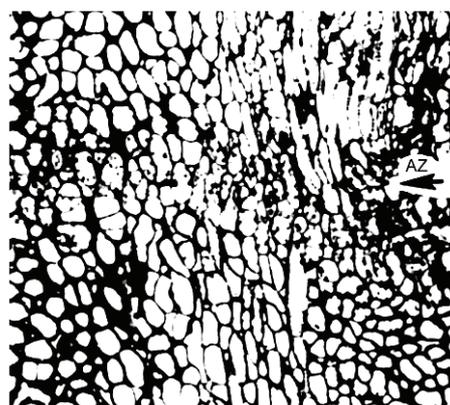


Fig. 1. Fruit abscission zone (AZ) of mature Valencia orange (Kazokas *et al.* 1998). The arrowhead points at AZ.

embryogeny and is synergistic or antagonistic gene interaction in AZ stringently controlled by a large number of genes in a temporal and spatial manner. However, the

network of development remains unclear. Besides a radiation- and a hormone-signalling pathway, other

signalling pathways related to AZ development still need to be discovered.

Compounds involved in fruit abscission

Hormones: Since plant hormones are involved in every aspect of plant biology, it is not surprising that a large number of genes regulating fruit abscission are part of the hormone biosynthetic and signalling pathways or exert their effects by influencing hormone metabolism. They often rapidly modify gene expression through inducing or repressing the degradation of transcriptional regulators *via* the ubiquitin-proteasome system (Ooms *et al.* 1993).

Ethylene is generally considered to play a crucial role in fruit abscission. Application of aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, and 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, obviously reduce fruit drop (Yuan and Li 2008). On the contrary, ethephon, an ethylene-releasing compound, effectively promotes the abscission of fruit (Zhu and Yuan 2010) indicating that both ethylene biosynthetic and signalling pathways are involved in fruit abscission. *ACS* (ACC synthase gene) and *ACO* (ACC oxidase gene) are well known to be ethylene biosynthetic genes. From pharmacological and gene expression analysis, the increased expression of these two genes concomitant with an increase in concentration of ethylene is well related to the fruit drop (Zhu *et al.* 2008, Botton *et al.* 2011, Kolarič *et al.* 2011, Parra-Lobato and Gomez-Jimenez 2011). Overexpressing the ethylene biosynthesis gene (*ACC*) in tomato plants resulted in premature flower abscission (Lanahan *et al.* 1994). Data mentioned above show that ethylene not only serves as a key factor responsible for fruit abscission directly or indirectly but also accelerates fruit abscission in a concentration-dependent manner. Both *ACS* and *ACO* belong to the multigene families of which only a small part of their members are related to fruit abscission. For instance, in apple, five *ACS* genes, *i.e.*, *MdACS1*, *MdACS2*, *MdACS3*, *MdACS5A*, and *MdACS5B* (Dal Cin *et al.* 2005) and four *ACO* genes, *i.e.*, *MdACO1*, *MdACO2*, *MdACO3*, and *MdACO4* (Wiersma *et al.* 2007) have been isolated and characterized. Among them, only *MdACS5A*, *MdACS5B*, and *MdACO1* are related to fruit abscission. These data suggest that ethylene biosynthetic genes responsible for fruit abscission should be specifically regulated by abscission-related signals. Recently, a number of ethylene receptors and downstream signal components related to fruit abscission have been identified in plants. ERSs and ETRs as ethylene receptors are specifically expressed in the AZ during fruit abscission and can be induced by ethephon (Ish-Shalom *et al.* 2011), propylene (Rasori *et al.* 2002), and naphthaleneacetic acid (NAA) (Zhu *et al.* 2008), but repressed as expected by 1-MCP (Rasori *et al.* 2002, Li *et al.* 2010) and AVG (Zhu *et al.* 2008) demonstrating

that ethylene signalling was involved in abscission process. A further support of this hypothesis is the fact that an ethylene downstream signalling gene, *EIL2*, is also related to fruit abscission as shown by the results of gene expression and genetic analysis (Parra-Lobato and Gomez-Jimenez 2011). Because of an inversed relationship existing between receptor abundance and ethylene sensitivity, the differences in the occurrence of ethylene receptors, such as ERSs and/or ETRs, could explain differential ethylene sensitivity in the AZ of a variety of plant species (Karupiah and Burns 2010). Therefore, the ERS/ETR ratio may be more suitable to serve as an indicator for abscission than does either ERS or ETR alone (Dal Cin *et al.* 2005). However, in *Arabidopsis* or tomato ethylene perception mutants, abscission still occurs albeit delayed (Lanahan *et al.* 1994, Bleecher and Patterson 1997, Whitelaw *et al.* 2002) indicating either ethylene plays a role in the timing of abscission or redundancy in ethylene perception genes may exist. Among natural apple variants, some of them do not show abscission in spite of high content of ethylene (Sun *et al.* 2009). In citrus, the induction of higher ethylene content after the period of the AZ activity was not able to promote fruit drop (Iglesias *et al.* 2006). Furthermore, 1-MCP appeared not to reduce fruit abscission (Pozo *et al.* 2004, Zhu *et al.* 2010). Taken together, all these data indicated that ethylene was not the only regulator of fruit abscission and there may be some ethylene-independent fruit abscission mechanisms.

Other hormones, particularly ABA, IAA, and GA also play substantial direct or indirect roles in fruit abscission. ABA is a synergist to ethylene through promoting the production of ACC. Under saccharide deficiency, water stress and wounding increase fruit abscission concomitantly with the accumulation of endogenous ABA (Gómez-Cadenas *et al.* 2000, Hilt and Bessis 2003, Yuan *et al.* 2003, Mahouachi *et al.* 2005, Iglesias *et al.* 2006, Zhu *et al.* 2011), however, application of exogenous ABA does not promote fruit abscission in intact plant (Gómez-Cadenas *et al.* 2000). It has been suggested that there should be a mechanism in plants that can sense abiotic stress and then adjust ABA homeostasis in response to environmental conditions which ultimately controls fruit abscission. To date, numerous genes related to ABA *de novo* biosynthesis and genes encoding receptors and downstream signal components have been characterized in *Arabidopsis thaliana* (McCourt and Creelman 2008, Peleg and Blumwald 2011) but in this regard, no reports related to fruit abscission are known.

Contrary to ABA, IAA flux across the AZ of fruit decreased the ethylene-sensitivity of this organ and

subsequently increased fruit retention (Bangerth 2000, Iglesias *et al.* 2006, Smith and Whiting 2010, Botton *et al.* 2011). AZ transcriptome analysis showed that auxin depletion resulted in changes in auxin-regulated gene expression related to the acquisition of ethylene sensitivity (Zhu *et al.* 2011). The reduced supply of auxin to AZ concurrently with a likely depolarization of its transport would enhance its sensitivity to ethylene and the consequent activation of cell wall-degrading enzymes (Sexton and Roberts 1982). It has been shown that polar auxin transport (PAT) was essential for fruit retention. Therefore, perturbations to PAT by 2,3,5-triiodobenzoic acid, a PAT inhibitor which blocks trafficking the member of the putative efflux carrier PIN1, obviously promotes fruit abscission (Else *et al.* 2004, Blanusa *et al.* 2005). Recently, several IAA efflux/influx carriers have been isolated and characterized, the same case to transcription regulators and IAA hydrogen symporters. AUX/IAA transcription regulators play a pivotal role in auxin perception and transduction. An AUX/IAA transcription regulator has been cloned by using degenerated primers from apple and its transcripts were induced by auxin but inhibited by ethylene (Dal Cin *et al.* 2007). Among IAA efflux/influx carriers, the expression level of *MdLAX1*, *MdPIN10*, and *MdPIN4* were considered to be involved into apple abscission with *MdPIN10* being repressed by ethylene (Dal Cin *et al.* 2009a). Using cDNA-AFLP approach, an auxin hydrogen symporter gene (*MdAHS*) has been isolated. This gene is characterized by the absence of auxin interacting regions and its transcripts are affected neither by auxin nor by ethylene but associated with fruit abscission indicating that it might interplay with other genes in the process of auxin transport and be regulated by other factors (Dal Cin *et al.* 2009b). These findings suggested that fruit abscission was determined possibly by cross-talk between IAA and ethylene in a similar way as mentioned in the previous report indicating that auxin might prevent the ethylene effect by interfering with the signalling process of ethylene (Taesakul *et al.* 2012).

Like IAA, nitric oxide (NO) represses ethylene production to prevent fruit from abscission by the binding NO to ACO forming a binary ACO-NO complex which is then chelated by ACC to produce a stable ACC-ACO-NO complex (Parra-Lobalo and Gomez-Jimenez 2011). Currently, it has been found that gibberellins (GAs) might enhance fruit retention (Mahouachi *et al.* 2009) maybe through accelerating IAA metabolism (Chen *et al.* 2006) or through enhancing the transport of saccharides. On the contrary, jasmonate (JA) is thought to initiate some abscission processes in an ethylene-independent manner by modifying saccharide metabolism in the AZ (Hartmond *et al.* 2000, Pozo *et al.* 2004). However, to our knowledge, both the mechanisms of JA and GA are still controversial. Collectively, afore-mentioned data show that the mechanisms controlling fruit abscission is still not clear and further investigations are needed.

Saccharides: Saccharides are essential for life on earth and serve not only as parts of metabolic pathways but also as signalling molecules (Hanson and Smeekens 2009). Saccharide shortage promotes fruit abscission concomitant with increase in ABA and 1-aminocyclopropane-1-carboxylic acid (ACC) concentrations (Iglesias *et al.* 2003). Through sucrose supplementation and branch girdling treatment, saccharose content was enhanced and subsequently increased fruit set but did not counteract the abscising effect induced by ACC (Iglesias *et al.* 2006). These results indicate that saccharides may act not only as essential nutrient but also as signalling molecules (Iglesias *et al.* 2003). It is also suggested that saccharides as signalling molecules control fruit abscission by affecting hormones metabolism and/or signalling pathways. However, flower and early fruit abscission is not affected by saccharides shortage (Ruiz *et al.* 2001). It is likely that the saccharide threshold level leading to fruit abscission is different at different development stages. Several genes sensing the content of saccharides and related to fruit abscission have been isolated and characterized. One of them is trehalose-6-phosphate synthase (*TPS*) belonging to trehalose metabolism genes. Since trehalose generally serves as a storage saccharide and stress protectant and usually accumulates under starvation conditions (Rolland *et al.* 2006, Fernandez *et al.* 2010), the involvement of *TPS* in abscission is suggested by the high expression in abscising fruitlet (Botton *et al.* 2011) and *Citrus* fruits (Alferez *et al.* 2007).

The *SnRK3*-like gene and the *SUS*-like Suc synthase gene are also believed to serve as signal molecules sensing nutrient storage and subsequently being involved into fruit abscission (Botton *et al.* 2011). Through gene expression and pharmacological analysis, the hexokinase gene (*HXK*) is up-regulated by NAA only in the AZ of apple suggesting that it is also probably involved in fruit abscission acting as a signal gene. Interestingly, the sorbitol-6-phosphate dehydrogenase genes, which plays a key role in the biosynthesis of sorbitol but is generally considered not to be expressed in young fruits, is obviously up-regulated after shade treatment. However, whether this gene is involved in fruit abscission needs further investigation (Zhou *et al.* 2008).

In addition to saccharide signalling genes as mentioned above, two saccharide transport genes, *i.e.*, the sorbitol and the sucrose transporter genes (*MdSOT* and *MdSUT*) have been characterized in detail by microarray analysis combined with gene expression methods. These two genes are both repressed in the fruit AZ by NAA and shading suggesting that the saccharide transport to fruitlets is hindered which subsequently leads to fruit abscission. All the findings mentioned above indicate that saccharides not only provide nutrition but also play a key role as signalling molecules and that the cross-talks between saccharides and hormones or other factors can effectively regulate fruit abscission. However, in some

cases, the hypothesis that saccharides acting as a signal molecule may be directly involved in fruit abscission regulation still needs future investigations.

Polyamines: Polyamines (PAs), mainly including putrescine (Put), spermidine (Spd), and spermine (Spm), are cationic compounds ubiquitously distributed in living organisms. They are also implicated in increasing fruit set (Albuquerque *et al.* 2006, Dal Cin *et al.* 2009a,b). In olive cultivars, the activities of diamine oxidase (DAO) and polyamine oxidase (PAO) are inversely associated with fruit abscission (Gomez-Jimenez *et al.* 2010). Using cyclohexylamine and β -hydroxyethylhydrazine as inhibitors for Spd synthase (SPDS) and PAO, respectively, resulted in the promotion of grapevine fruit abscission (Aziz *et al.* 2001). These data indicate that Put may play an important role in regulating fruit abscission. However, only soluble Put but not soluble Spd and Spm is involved in hindering fruit abscission and with a positive influence on fruit set (Aziz *et al.* 2001, Gomez-Jimenez *et al.* 2010). The mechanism behind the role of soluble and insoluble Put in fruit abscission may be that the conversion of free Put to conjugated Put could alleviate the wounding effect of free Put on membranes of plant cells and stabilize the configuration and function of proteins (Gomez-Jimenez *et al.* 2010). According to the above findings, the decrease in activities of PAO, DAO, and SPDS contributes to an increase in free Put content in response to fruit abscission. In addition to Put, other PAs, such as Spd and cadaverine (Cad), are also associated with fruit abscission (Gomez-Jimenez *et al.* 2010). S-adenosyl methionine (SAM) decarboxylase (*SAMDC*) is considered as a major regulatory gene involved in the synthesis of Spd and Spm and it is also known to affect the ethylene production due to the antagonistic relationships between PA and ethylene biosynthesis because both synthetic routes are competing for the utilization of SAM, a common precursor. During fruit abscission, *SAMDC* activity is inhibited while the

endogenous content of free Put and ethylene increases (Gomez-Jimenez *et al.* 2010), both phenomena contributing to fruit abscission. Additionally, PAs regulate ethylene biosynthetic and signalling genes such as *OeACS2*, *OeEIL2*, and they are related to NO and H₂O₂ productions, both molecules serving as signal molecules during fruit abscission (Gil-Amado *et al.* 2011, Parra-Lobato and Gomez-Jimenez 2011) indicating that PAs are implicated into signal transduction pathway related to fruit abscission.

Others: Lipids provide the structural basis for cell membranes and fuels for metabolism (Wang 2004). In recent years, accumulating evidences have been obtained indicating that lipids also function in signal transduction (Wang 2004). After application of 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMNP) to citrus, the phospholipase A₂ (PLA₂) and lipoxygenase (LOX) activities as well as lipid hydroperoxide content increased in flavedo of citrus fruit. On the contrary, aristolochic acid, a PLA₂ activity inhibitor, reduced the CMNP-induced increases in PLA₂ and LOX activities and lipid hydroperoxide levels concomitant with an obvious reduction in fruit abscission. These data indicate that lipids play important roles in fruit abscission (Alferez *et al.* 2005) although the mechanisms behind the sensing and transduction of the abscission signals remain obscure. Recently, a lipid transfer protein (*LTP*) gene has been isolated from mature citrus fruit by subtraction library. Through genetics and expression analysis, it revealed that the *LTP* gene appeared to play an important role in the fruit abscission possibly by assisting in the transport of cutin monomers to the fracture plane of the AZ or through its antimicrobial activity by reducing the potential of microbial attack (Wu and Burns 2003). Collectively, in addition to the aforementioned biological pathways, other mechanisms may be also involved into fruit abscission which remains to be explored further.

Hormone, saccharide and polyamine cross talk

Accumulating evidences supporting the cross-talk among hormones, between hormones and saccharides, as well as between hormones and polyamines are mainly obtained from research on apple, mango, and citrus. The synergistic or antagonistic action of these compounds and the coordinated regulation of their corresponding biosynthetic pathways play important roles in modulating fruit abscission enabling adequate response to internal and external factors (Fig. 2). Nowadays, it is generally accepted that a balance between ethylene and auxin content at the AZ is a crucial factor regulating fruit abscission. Ethylene promotes fruit abscission whereas auxin hinders this process and also reduces the sensitivity of the AZ to ethylene (Taylor *et al.* 2001). However,

auxin itself stimulates ethylene production by increasing the expression of ACS genes (Vandenbussche and Straiten 2007, Li and Yuan 2008). In turn, ethylene serving as feedback inhibitor blocks auxin transport from fruit (Zhu *et al.* 2011). Detailed understanding the molecular events regarding the interplay between auxin and ethylene remains elusive. ABA appears to have an abscission accelerating effect by increasing in ACC levels (Bangerth 2000, Gómez-Cadenas *et al.* 2000). Therefore, fruit abscission in a few instances is determined by the relative concentrations of IAA and ABA (Racskó *et al.* 2006). To our knowledge, it is still unclear whether ABA is able to initiate fruit abscission directly. ABA might be involved in the sensing of saccharide deficiencies and

then link saccharide shortage to fruit abscission (Gómez-Cadenas *et al.* 2000).

Based on transcriptomic data, reactive oxygen species (ROS) signalling may be implicated in saccharose shortage and ABA signalling may concurrently orchestrate saccharose-ROS cross talk (Botton *et al.* 2011). Subsequently, ABA-ethylene cross talk triggered by related proteins may lead to fruit abscission. A gene encoding an AMP-activated protein kinase may be involved into ABA-saccharose cross talk which is found to be up-regulated in the abscising fruits (Botton *et al.* 2011). Another gene, D-sorbitol-6-phosphate dehydrogenase gene (*S6PDH*), maybe acts as an ABA-mediated stress response gene implicated in ABA-saccharose cross talk (Zhu *et al.* 2011). The responsible genes in cross talk between saccharides and hormones related to fruit abscission controlling need further identification. Especially, the role of saccharides as signal molecules in the direct regulation of hormone biosynthetic or in the

fruit abscission signalling pathways remains to be tested.

During olive fruit abscission, a rise in ethylene production was accompanied by free Put accumulation with an up- and a down-regulation of ADC and SAMDC activities, respectively, pointing at a relationship between the ethylene and the PA biosynthetic pathways which coordinately control fruit abscission (Gomez-Jimenez *et al.* 2010, Parra-Lobato and Gomez-Jimenez 2011). ADC and ODC activities were both up-regulated by exogenous ethylene although only ADC activity was inhibited by CoCl₂, an ACO inhibitor associated with fruit abscission, suggesting Put synthesis *via* ADC is regulated by ethylene mainly by stimulating ACO activity in the fruit AZ (Gil-Amado *et al.* 2011). In addition, CoCl₂ raised Spd and Spm amounts in the fruit AZ through enhancing SAMDC activity resulting in an enhanced flux of SAM, however, exogenous ethylene down-regulated SAMDC activities as well as *OeSAMDC1* gene expression (Gil-Amado *et al.* 2011).

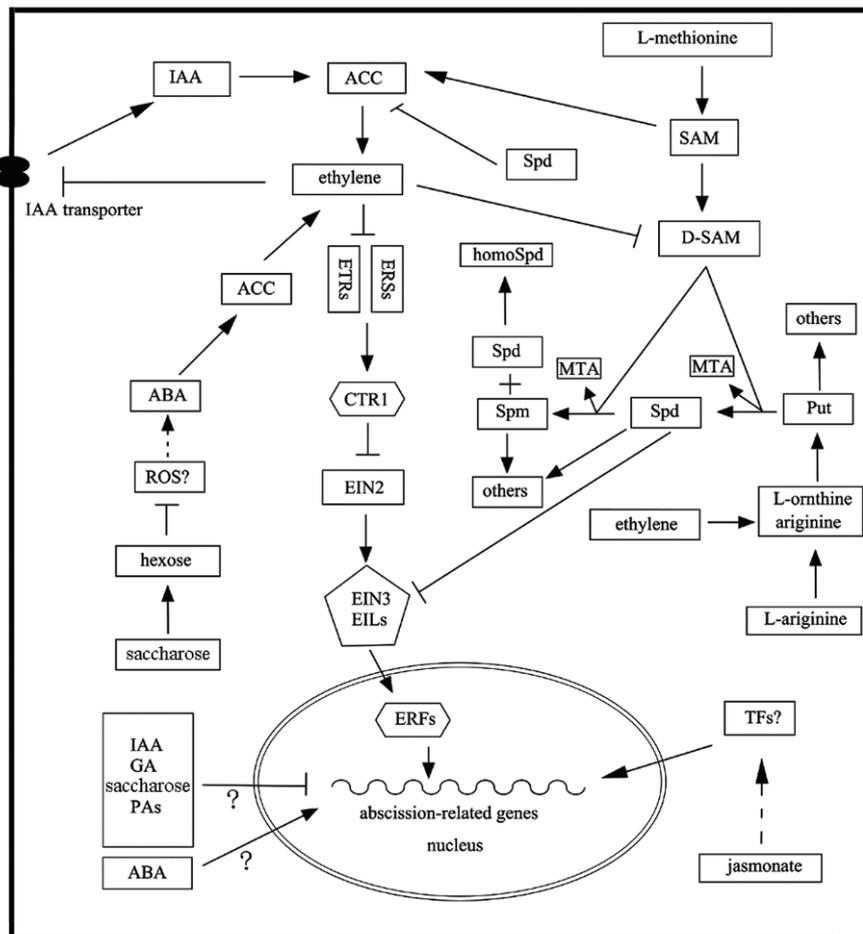


Fig. 2. Integration of fruit abscission mechanisms. Ethylene plays a pivotal role in facilitating fruit abscission. Auxin and ethylene maintain an important balance in the abscission zone by feedback regulating mechanisms. A second mechanism of fruit abscission is through the shortage of saccharides which enhances ABA content. ABA and ethylene are synergistic during fruit abscission. The interrelationship between the ethylene and the PA biosynthetic pathways, which coordinately control fruit abscission, is adjusted by SAMDC. Jasmonate leads to fruit abscission through an ethylene-independent mechanism. IAA, ABA, GA, PAs, and saccharose are suggested to be involved directly in regulating fruit abscission.

Given that ethylene and PA biosynthetic pathways are linked through SAM, it appeared that the antagonistic relationships between ethylene and PAs are chiefly adjusted by SAMDC activity (Gil-Amado *et al.* 2011) and that all the internal and external factors that can affect ethylene and PAs biosyntheses have great potential for altering SAMDC activity. Additional support for

indicating the competition between these two pathways comes from the observations that *OeACS2* and *OeEIL2* expressions are under the negative control of Spd whereas exogenous ET induces their expression during mature fruit abscission in olive (Parra-Lobato and Gomez-Jimenez 2011).

Cell wall degradation

The compounds composing cell wall in AZ, such as cellulose and pectin, have to be decomposed before fruit abscission, which is the final part of the fruit abscission developmental programme, and characterized by increased gene expression and enzyme activity of cell wall hydrolases including polygalacturonase (PG), β -1,4-glucanase (EG), β -galactosidase, expansin, and pectate lyase (Wu and Burns 2004, Zhu *et al.* 2011). Of these cell wall hydrolases, PG and EG are the main enzymes implicated into the fruit abscission in several species. They are directly regulated by ethylene (Kazokas and Burns 1998, Wu and Burns 2004, Li *et al.* 2010). However, no information, to the best of our knowledge, is available about whether other hormones, saccharides, PAs, and

other metabolites directly regulate these two genes. PGs and EGs have been isolated and characterized and shown to belong to a multigene family. For instance, in apple, only one member of PG gene family, *i.e.*, *MdPG2* but not *MdPG1* is associated with fruitlet abscission (Li and Yuan 2008, Zhu *et al.* 2008, 2011); similarly, only *MdEG1*, a member of *MdEGs*, is involved in fruit abscission. Interestingly, *MdPG2* expression can be enhanced by NAA in the fruitlet AZ but is inhibited in the mature fruit AZ which needs further investigation (Zhu *et al.* 2008). Maybe, the diversification in function of gene family members meets well with the demand of the spatial and/or temporal regulation making plants more suitable to survive.

Conclusions and perspectives

In recent years, the molecular mechanisms regulating fruit abscission have been at least partly elucidated and the roles of plant hormones, saccharides, and PAs in fruit abscission have been demonstrated. These findings might contribute to the modification of fruit abscission-related biosynthetic pathways aiming at the generation of transgenic plants in order to meet different requirements of producers. Mechanisms of fruit abscission are multiple and complex. A large body of equivalent studies is currently going on and seems to reveal additional complexities in the mechanism. Hormones play a crucial role in determining fruit abscission and have been most comprehensively studied but identifying their receptors and signalling components related to fruit abscission

remains a challenge. Saccharides and PAs as well as other metabolites regulate fruit abscission either through altering the hormone dose/response ratio or through direct involvement or in combination. Hexoses such as trehalose and glucose may serve as signal molecules that directly regulate the biosynthesis of hormones. Similarly, PAs directly affect the ethylene production and subsequently inhibit fruit abscission. However, a large number of genes involved in this process still need to be isolated and analyzed. Finally, the use of promoters driving fruit abscission-related gene expression under specific external stimuli or at specific development stages will facilitate to unravel the mechanism of fruit abscission.

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