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Research article

Distinct organ-specific and temporal expression profiles of auxin-related genes during mango fruitlet drop

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ABSTRACT

In mango, fruitlet abscission initiates with a decrease in polar auxin transport through the abscission zone (AZ), triggered by ethylene. To explore the molecular components affecting this process, we initially conducted experiments with developing fruitlet explants in which fruitlet drop was induced by ethephon, and monitored the expression patterns of distinct indole-3-acetic acid (IAA)-related genes, comparing control vs. ethephon-treated pericarp and AZ profiles. Over the examined time period (48 h), the accumulation of *MiPIN1* and *MiLAX2* IAA-efflux and influx genes decreased in both control and treated tissues. Nevertheless, ethephon-treated tissues displayed significantly lower levels of these transcripts within 18–24 h. An opposite pattern was observed for *MiLAX3*, which overall exhibited up-regulation in treated fruitlet tissues. Ethephon treatment also induced an early and pronounced down-regulation of five out of six IAA-responsive genes, and a substantial reduction in the accumulation of two IAA-synthesis related transcripts, contrasting with significant up-regulation of *Gretchen Hagen3* transcript (*MiGH3.1*) encoding an IAA–amino synthetase. Furthermore, for both control and treated AZ, the decrease in IAA-carrier transcripts was associated with a decrease in IAA content and an increase in IAA–Asp:IAA ratio, suggesting that fruitlet drop is accompanied by formation of this non-hydrolyzed IAA–amino acid conjugate. Despite these similarities, ethephon-treated AZ displayed a sharper decrease in IAA content and higher IAA–Asp:IAA ratio within 18 h. Lastly, the response of IAA-related genes to exogenous IAA treatment was also examined. Our results are discussed, highlighting the roles that distinct IAA-related genes might assume during mango fruitlet drop.

1. Introduction

In plants, abscission of reproductive structures has developed to facilitate the shedding of excess, damaged, or no longer necessary organs. In particular, fruitlet abscission occurring during fruit development is characterized by activation of a pre-differentiated abscission zone (AZ), located between the pedicel and fruitlet (Bonghi and Ramina, 2000; Sawicki et al., 2015). The main hormones controlling AZ formation are ethylene, acting as an inducer, and auxin (indole-3-acetic acid, IAA), acting as a suppressor. As such, diffusion of ethylene from fruit tissues to the AZ is suggested to play a role in initiating abscission events by accelerating the activities of cell-wall-degrading enzymes at the AZ (Bonghi and Ramina, 2000; Sawicki et al., 2015). On the other hand, basipetal IAA flux though the AZ is thought to inhibit abscission by reducing the sensitivity of the AZ area to ethylene (Estornell et al., 2013; Sawicki et al., 2015).

Auxin plays an important role in controlling many aspects of plant development (Vanneste and Firml, 2009). In particular, the balance of IAA synthesis, conjugation, degradation and transport is tightly regulated, leading to establishment of IAA homeostasis (Woodward and Bartel, 2005; Zhao, 2010). In principle, *de-novo* IAA synthesis, resulting from tryptophan (Trp)-dependent or independent pathways, is followed by its conjugation to amino acids, sugars and methylesters. Up to date, different pathways have been proposed to be involved in the production of IAA in plants. However, it has only recently been established that a simple two-step pathway,

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which includes the sequential action of two groups of enzymes - TAA1/TARs (TRYPTOPHAN AMINOTRANSFERASE of *ARABIDOPSIS1*/TRYPTOPHAN AMINOTRANSFERASE RELATED) and YUCCAs (flavin monooxygenases), acts as the main route contributing to the synthesis of free IAA (see reviewed in (Korasick et al., 2013; Ludwig-Müller and 2011; Zhao, 2010)). Following biosynthesis and conjugation, release of IAA from its conjugates is achieved by hydrolytic cleavage (Korasick et al., 2013; Ludwig-Müller, 2011). Different IAA conjugates have been identified. Of these, IAA–alanine (Ala), IAA–leucine (Leu) and IAA–phenylalanine (Phe), among others, have been shown to hydrolyze back to free IAA. In contrast, IAA–aspartate (Asp) and IAA–glutamate (Glu) are thought to serve as precursors for IAA degradation, as there is no evidence indicating that they can be hydrolyzed back to IAA (Ludwig-Müller, 2011). The transport of free IAA from sites of synthesis to target cells is further facilitated by influx and efflux carriers. Specifically, IAA efflux is achieved by active transport mediated by members of the PIN-FORMED family of efflux carriers (PINs) (Petrasek and Friml, 2009), whereas IAA import is facilitated by AUXIN RESISTANT 1/LIKE AUXIN RESISTANT (AUX1/LAX) family members, (Peret et al., 2012). After reaching the target tissues, IAA perception and signaling seems to depend on two types of receptors: ABP1 (AUXIN-BINDING PROTEIN 1) and TIR1/AFB (TRANSPORT INHIBITOR RESISTANCE 1/AUXIN SIGNALING F-BOX-TYPE) (Chapman and Estelle, 2009). While the role of ABP1 in mediating auxin responses is still controversial (Gao et al., 2015), the signaling pathways involving TIR1/AFBs have been well characterized (Chapman and Estelle, 2009; Weijers and Wagner, 2016). Briefly, three families of early auxin-responsive genes, including *Aux/IAA*s encoding nuclear transcriptional repressors, *Gretchen Hagen3s* (*GH3*s) encoding IAA-conjugating enzymes, and small auxin-upregulated proteins (*SAUR*s) encoding short-lived proteins that likely function in cell expansion, all contain one or more binding motifs to auxin response factor (ARF) transcription factors in their promoter regions. Whereas at low IAA concentrations, a heterodimer of an ARF and an Aux/IAA represses transcription, at higher IAA concentration, IAA binds to TIR1/AFB, triggering the degradation of Aux/IAAs, releasing ARF transcription factors and leading to activation of the early IAA-response genes (Chapman and Estelle, 2009). It should be noted, however, that ARFs may also function as transcription factors that mediate repression of gene expression, depending on the amino acid composition of their middle region (Weijers and Wagner, 2016).

Over the last decade, knowledge has been gained on the functions of distinct IAA-related genes during reproductive organ abscission (Estornell et al., 2013; Sawicki et al., 2015). Moreover, recent molecular studies performed during flower abscission in tomato (Meir et al., 2010) and during fruitlet drop in apple (Botton et al., 2011; Dal Cin et al., 2009), citrus (Cheng et al., 2015), litchi (Li et al., 2015) and grapevine (Kühn et al., 2016) have shown that acquisition of ethylene sensitivity in the AZ is associated with, among other factors, disruption of IAA homeostasis and altered expression of distinct IAA-related genes. Interestingly, some of these studies reported that similar but not identical changes in expression patterns of IAA-related genes occur in both reproductive organs that are about to abscise and in their AZ (Kuang et al., 2012; Meir et al., 2010, 2015).

Mango (*Mangifera indica* L.) is a very important tropical fruit crop. Despite satisfactory flowering and fruit set, mango production does not meet its potential due to intense natural fruitlet drop, leading to losses in revenue (Singh et al., 2005). Abscission events take place in mango during three distinct fruit-development stages: the first 2 months after fruit set, the mid-season (when fruit are 60–75 days old), and just before fruit maturity. Nevertheless, the highest abscission rates are observed during the early stages of fruit development, followed by gradually less, albeit still intense, fruitlet drop rates as the fruit reach maturity (Nunez-Elisea and Davenport, 1986; Singh et al., 2005). Interestingly, mango studies have shown that natural fruitlet drop correlates with enhanced endogenous ethylene production in both seed and pericarp tissues, however, pedicels containing the AZ produced no detectable ethylene prior to or at the moment of abscission (Nunez-Elisea and Davenport, 1986). To gain preliminary insight into the molecular mechanism regulating mango fruitlet drop, we previously cloned a mango gene encoding an ERS1-type ethylene receptor and its expression was monitored during fruitlet drop and fruit ripening (Ish-Shalom et al., 2011). Our data highlighted *MiERS1's* function in regulating fruitlet abscission (Ish-Shalom et al., 2011), a conclusion that was also recently corroborated in a study by Hagemann et al. (2015). Notably, in the latter study, it was also determined that mango fruitlet drop is associated with a reduction in polar auxin transport capacity through the fruitlet pedicel, although the biological events controlling this process were not elucidated (Hagemann et al., 2015).

Here, to determine whether mango fruitlet drop is accompanied by specific deactivation or activation of distinct IAA-related genes, experiments were first conducted with developing fruitlet explants in which fruitlet abscission was induced by the ethylene-releasing compound ethephon. The spatial and temporal expression patterns of selected IAA-related genes were investigated by comparing their expression profiles in control vs. ethephon-treated fruitlet tissues. The study was next expanded to quantify free IAA and IAA conjugates in control and ethephon-treated AZ tissues and to explore the response of IAA-related genes to exogenous treatment with synthetic IAA. Our results highlight the potential roles of distinct IAA-related genes in decreasing IAA levels and increasing the IAA–Asp:IAA ratio in the AZ, thus affecting mango fruitlet drop.

2. Materials and methods

2.1. Plant material, induction of fruitlet abscission by ethephon, and auxin treatment

Mango (*Mangifera indica* L.) explants were collected in the spring from commercially bearing 'Kent' trees grown in Ramot, in northeast Israel, on 12 May 2014, and on 07 May, 2016. The explants, each bearing 1 to 2 fruitlets per panicle (approx. 20 cm long), were kept in water and brought to the laboratory within 2.5 h of collection. In the laboratory, the explants were divided into six (May, 2014) or nine (May, 2016) experimental units, each composed of 110–120 explants. The basal end of each explant was placed in a 50-mL tube containing water and kept at 25 °C. On May, 2014, fruitlet abscission was induced in three experimental units using ethephon (Ethrel, 1.4 g L^{-1}, Agan Chemicals, Ashdod, Israel), as previously described (Ish-Shalom et al., 2011). The remaining untreated experimental units served as controls. On May 2016, fruitlet abscission was induced in three experimental units using ethephon, three experimental units were sprayed with 2,4-dichlorophenoxyacetic acid (2,4-D) (Hadranol™, 0.2 g L^{-1}, Machteshim, Beer Sheva, Israel), and the remaining experimental units served as controls. A non-ionic surfactant, Triton X-100, was included in all sprays at 0.025%. In both occasions, samples from the AZ and fruitlet pericarp of treated and control experimental units were collected at different time points, frozen in liquid nitrogen and kept at −80 °C until further analysis.

2.2. RNA isolation and cDNA synthesis

Plant tissues were ground in liquid nitrogen using an IKA-A11 analytical grinding mill (IKA®-Werke GmbH & Co. KG, Staufen,