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Hormonal interactions during early physiological partenocarpic fruitlet abscission in persimmon (*Diospyros Kaki* Thunb.) 'Triumph' and 'Shinshu' cultivars

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ABSTRACT

Fruitlet abscission is typical to many fruit trees, including persimmon, (*Diopyros kaki* Thunb.), and often results in unstable fruit production and yield. We documented massive parthenocarpic fruitlet abscission in the persimmon cultivars 'Triumph' and 'Shinshu', which initiated soon after anthesis and peaked at 45 days post anthesis. In contrast, abscission of mature fruitlets 45–65 days after anthesis was minor. The early parthenocarpic fruitlet abscission in 'Shinshu' and 'Triumph' took place in the abscission zone located at the calyx-pedicel junction. Differentiation of the abscission layer occurred during flower development and preceded fruitlet abscission. Endogenous analysis of gibberellins, cytokinins and auxin levels in the abscission zone of 'Triumph' and 'Shinshu' during the developmental stages preceding abscission revealed a reduction in cytokinin endogenous levels in both cultivars. In addition, a decline in GA_3 and IAA levels was found in 'Triumph' and 'Shinsu' respectively during flower development. Gibberellin, auxin and cytokinin application to inhibit early fruitlet abscission were analyzed and gibberellin treatment exhibited the highest and most consistent inhibition effect in 'Triumph' and 'Shinshu'. An increase in endogenous levels of gibberellins, cytokinins and auxin in the flower abscission zone was found following GA application in both cultivars. Our results suggest that gibberellin application inhibits early fruitlet abscission by elevating cytokinins, and auxin levels and that a reduction in gibberellins, cytokinins and auxin levels may induce abscission during parthenocarpic fruitlet development in 'Shinshu' and 'Triumph' cultivars.

1. Introduction

Plants shed various organs throughout their life cycle in an active process termed abscission. Abscission is an important and valuable trait in modern agriculture. The ability to inhibit abscission in order to reduce shedding of seeds, flowers, fruitlets or fruit can contribute to higher yield (Nakano and Ito, 2013; Patterson et al., 2016; Roberts et al., 2002). Abscission takes place in a designated tissue called the abscission zone, which is composed of several cell layers located between the abscised organ and the plant body (Osborne and Morgan, 1989; Roberts et al., 2002; Sexton and Roberts, 1982). The abscission layer is differentiated during organ development and activated by hormonal signals which initiate cell separation in the abscission zone (Osborne and Morgan, 1989). Studies in different plant species and various abscission processes showed that ethylene acts as the major hormone promoting abscission while auxin acts as the main hormone inhibiting the process. However many studies also showed that hormonal regulation of abscission is a complex interplay between different hormones that varies between distinct tissues and is dependent upon the developmental stage (Kim, 2014; Osborne and Morgan, 1989; Taylor and Whitelaw, 2001).

Physiological fruitlet drop is typical to many fruit trees, and often results in unstable fruit development and yield (Arseneault and Cline, 2016; Garner and Lovatt, 2016; George et al., 1997). Enhanced fruitlet abscission was found to occur during parthenocarpic fruit set, which is the development of the ovary into a seedless fruit in the absence of pollination and/or fertilization, an attractive trait leading to the production of seedless fruits (George et al., 1997; Gorguet et al., 2005). In tomato fruits, a model used to study parthenocarpy (Mariotti et al., 2011; Vriezen et al., 2008), studies showed that pollination and seed formation trigger fruit development by upregulating gibberellin and auxin biosynthesis and signaling (Fos et al., 2000, 2001; Olimpieri

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et al., 2007, Serrani et al., 2007). Application of gibberellin and auxin can substitute for pollination and/or fertilization and result in parthenocarpic tomato fruit development (Fos et al., 2001; Serrani et al., 2007). Cytokinin was also shown to induce parthenocarpic fruit growth in tomato and suggested to upregulate endogenous levels of gibberellin and auxin (Ding et al., 2013). The role of gibberellin in inhibiting abscission during fruit development was demonstrated in citrus (Ben-Cheikh et al., 1997; Talon et al., 1992). Self-incompatible seedless varieties exhibited low fruit set and intense fruitlet abscission, in the absence of cross-pollination, which were reduced by applications of gibberellin. Endogenous gibberellins were suggested to serves a similar function in non-parthenocarpic fruit as pollination was found to upregulate endogenous levels of gibberellin during fruit development in citrus seeded varieties (Ben-Cheikh et al., 1997).

In persimmon, (*Diopyros kaki* Thunb.) parthenocarpic fruitlet abscission is characterized by two main waves of fruit drop, with the first wave taking place soon after flower anthesis (early fruitlet drop) and the second occurring at later stages of fruitlet growth (George et al., 1997; Mowat et al., 1995). Pollinated and seeded fruit exhibited reduced fruit drop relative to parthenocarpic fruits (George et al., 1993, 1995; George et al., 1997; Woodburn and Andersen, 1996). Plant growth regulators were found to have a positive effect on parthenocarpic fruit set (George et al., 1997). Application of gibberellin was reported to substitute pollination and retard early fruitlet drop in different parthenocarpic cultivars (Blumenfeld, 1981; Yamamura et al., 1989). Treatment with cytokinin improved fruit set and exhibited higher inhibition of abscission than hand-pollination in 'Matsumoto-Wase-Fuyu' (Sugiyama and Yamaki, 1995).

The enhanced fruitlet abscission in modern seedless persimmon varieties requires agricultural practice to allow high efficiency of parthenocarpic fruit development. However, despite the accumulative studies, the regulation of persimmon parthenocarpic fruitlet abscission is still ambiguous, limiting the development of an effective treatment to inhibit abscission. The aim of the present study was to gain insights into the hormonal mechanisms involved in early physiological fruitlet abscission during parthenocarpic fruit set in the astringent 'Triumph' and non-astringent 'Shinshu' cultivars.

2. Material and methods

2.1. Plant material

Experiments were conducted in an orchard at the Volcani Center, ARO, Rishon Lezion, Israel. Triumph and Shinshu cultivars grafted on *Diospyros virginiana* L. seedling rootstocks were planted in 2007, at a spacing of 5 m × 5 m. The orchard consists of female trees with parthenocarpic fruit set.

2.2. Growth regulator experiments

Auxin, cytokinin and gibberellin treatments – The experiment was designed as a randomized block with 5 replication. Seven bearing branches were tagged on five different trees from each cultivar ('Triumph' and 'Shinshu'). The following treatments were sprayed to run-off: 1) Control - 0.025% Triton X-100 2) 10 mg l^{-1} 2,4-D (Hadranol, Adama, Israel) 3) 20 mg l^{-1} 2,4-D 3) 5 mg l^{-1} 1-(2-chloro-4-pyridyl)-3-phenylurea (CPPU) (AlzChem, Germany) 4) 10 mg l^{-1} CPPU 5) 30 mg l^{-1} gibberellin (GA_{3+1}, 1:20 ratio) (Sigma-Aldrich, USA) 6) 100 mg l^{-1} GA_{3+1}. Solutions 2–6 contained 0.025% Triton X-100, as a wetting agent. Fruit abscission was calculated before the treatments and 45 days after spraying and abscission rate was calculated as the percentage of abscised fruitlets.

Gibberellin (GA) treatment - The experiment was designed as a randomized block with 5 replication. Five whole trees from each cultivar were sprayed with 100 mg l^{-1} GA_{3+1} to run-off. Fruit abscission was calculated before the treatments, 45 and 65 days after spraying and abscission rate was calculated as the percentage of abscised fruitlets.

2.3. Histology analysis

'Triumph' and 'Shinshu' buds and flowers were removed and fixed overnight in 4% paraformaldehyde (PFA) (v/v), rinsed in PBS buffer and dehydrated through an ethanol series up to 95% ethanol. Fixed and dehydrated issues were infiltrated with catalyzed monomer A of the JB-4 embedding kit (Electron Microscopy Sciences) and embedded into JB-4 plastic resin under an oxygen-free environment. Blocks were serially sectioned (interval 4 mm) on a rotary microtome (Leica RM2255) with TC-65 disposable blades (Leica). Sections were stained with 0.1% (w/v) toluidine blue prior to examination and photographed on an Olympus BX53 digital microscope equipped with an Olympus DP73 digital camera using bright field.

2.4. Hormone analysis using LC–MS-MS

Hormone extraction was performed using standard protocols (Müller and Munné-Bosch, 2011) with slight modifications. Briefly, frozen tissue was grinded to a fine powder using a mortar and pestle. 200 mg of the powder was transferred to a 2 ml tube containing 1 ml extraction solvent (ES) mixture (79% IPA: 20% MeOH: 1% acetic acid) supplemented with 20 ng of each deuterium-labelled internal standards (IS, Olomouc, Czech Republic). The tubes were incubated for 60 min at 4 °C with rapid shake and centrifuged at 14,000 g for 15 min at 4 °C. The supernatant was collected and transferred to 2 ml tube. 0.5 ml of ES was added to the pellet and the extraction steps were repeated twice. The combined extracts were evaporated using speed-vac at RT. Dried samples were dissolved in 200 µl 50% methanol and filtered with 0.22 µm PVDF syringe filter. 5–10 µl were injected for each analysis.

LC–MS-MS analyses were conducted using UPLC-Triple Quadrupole-MS (Waters Xevo TQ MS). Separation was performed on Waters Acquity UPLC BEH C18 1.7 µm 2.1 x 100 mm column with a VanGuard pre-column (BEH C18 1.7 µm 2.1 x 5 mm).

Chromatographic parameters - The mobile phase consisted of water (phase A) and acetonitrile (phase B), both containing 0.1% formic acid in the gradient elution mode.

Solvent gradient program for auxins and cytokinins:

Time (min)	Phase A %	Phase B %
Initial	95	5
0.5	95	5
14	50	50
15	5	95
18	5	95
19	95	5
22	95	5

Solvent gradient For GAs analysis:

Time (min)	Phase A %	Phase B %
Initial	95	5
0.5	95	5
12	50	50
13	5	95
16	5	95
17	95	5
20	95	5

The flow rate was 0.3 ml/min, and the column temperature was