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## RESEARCH PAPER

# Immature fruit abscission is triggered by hormonal changes in the seed coat of avocado

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Received 23 March 2026; Accepted 1 June 2026

Editor: Fabrizio Costa, University of Trento, Italy

## Abstract

**Avocado (*Persea americana*) is an economically important tree crop that exhibits a high rate of immature fruit abscission (IFA), reducing yield. As the seed coat derived from recently abscised fruitlets displays a senescence phenotype, we hypothesized that this maternal organ plays a critical role in initiating IFA. Here, we show that fruitlets fated to abscise undergo growth arrest before shrinking and detaching from the tree. Comparative RNA-seq analysis indicated that growth arrest is associated with a transcriptome reprogramming that is first initiated in the seed coat and then transmitted to pericarp and embryo. Gene-expression and hormone-profiling results indicated that fruitlet growth arrest is associated with a decline in auxin activity and an increase in abscisic acid, the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid, and the bioactive jasmonate jasmonoyl-isoleucine in the seed coat. At a late stage of growth arrest, transcriptomic signatures further suggested that a dormancy-like program of development is induced in the seed and a senescence phenotype is activated in the seed coat. Together, our data indicate that avocado IFA is initiated by hormone-driven transcriptome reprogramming that functions to transition the seed coat to a senescence program of development, which induces growth arrest, seed dormancy, and ultimately fruitlet abscission.**

**Keywords:** Absciscic acid, auxin, avocado, ethylene, fruit abscission, jasmonic acid, *Persea americana*, seed coat, seed dormancy, senescence.

## Introduction

Plant organs, including fruit, follow a highly regulated developmental sequence that begins with growth followed by maturation and culminates with senescence (Fenn and Giovannoni, 2021; Pautot *et al.*, 2025). The growth phase is

Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; AZ, abscission zone; DAFB, days after full bloom; DEG, differentially expressed gene; DEGA, defoliated–early growth arrest; DLGA, defoliated–late growth arrest; DPA, dihydrophaseic acid; ET, ethylene; IAA, indole-3-acetic acid; IAA-Asp, IAA-aspartate; IFA, immature fruit abscission; JA, jasmonic acid; JA-Ile, (+)-7-iso-jasmonoyl-L-isoleucine; NGA, natural growth arrest; PA, phaseic acid.

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characterized by active patterns of cell division and expansion. During maturation, cell differentiation is activated to establish specialized cells and tissues essential for organ function. As organs age, they undergo senescence, a programmed degenerative process frequently associated with abscission (Patharkar and Walker, 2019; Pautot *et al.*, 2025). In addition to age-related senescence, environmental stress, pathogen attack, and nutrient deficiency can also trigger a programmed degeneration process, often leading to the detachment of both mature and immature organs.

In many economically important fruit tree crops a substantial proportion of immature fruit undergo abscission during the growth phase of development (Sawicki *et al.*, 2015; Sadka *et al.*, 2023). It is hypothesized that immature fruit abscission (IFA) results from feedback interactions between expanding vegetative shoots and developing fruit, as well as among fruitlets (Sawicki *et al.*, 2015; Sadka *et al.*, 2023). According to this model, vegetative shoots and fruitlets with greater growth potential induce abscission in fruitlets with a lower growth potential. In apple (*Malus domestica*), applying fruitlet-thinning agents causes a large population of immature fruit to abscise, most likely via enhanced feedback interactions among the developing fruitlets (Botton and Ruperti, 2019). Further, after application of thinning agents, experimental studies suggest that fruitlets fated to abscise undergo growth arrest prior to abscission (Ward and Marini, 1999; Greene *et al.*, 2013). Thus, it appears that the primary effect of these feedback interactions is to inhibit growth before abscission is activated in the pedicel.

In developing fruit auxin is primarily produced in seeds, where it promotes growth and coordinates seed and fruit development (Kumar *et al.*, 2014; Figueiredo and Köhler, 2018; Fenn and Giovannoni, 2021). It is generally accepted that polar transport of auxin from the developing fruitlet through the pedicel suppresses the activation of the abscission zone (AZ) (Sawicki *et al.*, 2015; Pautot *et al.*, 2025); however, if the level of auxin is reduced in the AZ by a disruption in this transport system, cell separation can be activated by ethylene (ET) (Sawicki *et al.*, 2015; Pautot *et al.*, 2025). Consequently, intra-organ signaling events initiated within fruitlets fated to abscise are predicted to influence auxin production in the seed and/or its export through the pedicel, thereby permitting the activation of the AZ (Sawicki *et al.*, 2015; Botton and Ruperti, 2019).

Apple has served as a model system for investigating intra-organ signaling events associated with IFA (Sawicki *et al.*, 2015; Botton and Ruperti, 2019), with studies indicating that fruitlet abscission is initiated in the cortex by increased production of ET and abscisic acid (ABA) (Botton *et al.*, 2011). ET is proposed to act as a mobile signal that diffuses from the cortex to the seed, where it induces abortion (Eccher *et al.*, 2015). Seed abortion is thought to reduce auxin transport through the pedicel, thereby enabling ET to activate cell separation processes in the AZ (Sawicki *et al.*, 2015; Botton and Ruperti, 2019). The role of ABA is less clear as it has been

speculated that its production in the cortex is due to a stress response and might not play a direct role in initiating embryo abortion (Botton and Ruperti, 2019). Although apple provides a valuable framework for understanding IFA, it remains unclear whether the signaling mechanism(s) that functions upstream of AZ activation is conserved across different fruit tree species.

Avocado (*Persea americana*) exhibits a high rate of IFA starting in spring within 2 weeks after flowering, which continues through the summer before declining in autumn (Salazar-Garcia *et al.*, 2013). A substantial proportion of fruitlets that abscise during the first 2–3 weeks after flowering are unfertilized, whereas those that abscise after ~4 weeks are typically fertilized (Sedgley, 1980; Garner and Lovatt, 2016). Notably, fruitlets that drop during summer that exceed 20 mm in diameter display a premature senescence phenotype in the seed coat (Garner and Lovatt, 2016). Experimental observations show that weakly attached fruitlets that are probably in the final stage of abscission are generally smaller than persisting fruitlets, suggesting that growth arrest might precede abscission. The presence of a premature senescence phenotype in the seed coat of weakly attached fruitlets further indicates that degeneration of the seed coat might contribute to the initiation of avocado IFA (Garner and Lovatt, 2016).

The hormonal control of avocado IFA has been investigated using two complementary approaches. In one study, the role of ET was examined in fruitlets on branches excised from trees when they were between 10–25 mm in diameter (Davenport and Manners, 1982). Under these conditions, IFA was initiated ~3 d after branch excision. Tissue degeneration within the nucellus and seed coat was observed within 2 d, coinciding with a marked burst in ET production primarily in the seed coat. In separate studies conducted during the summer-drop period, weakly attached fruitlets that were probably in the final stage of abscission contained higher levels of ABA and produced more ET than persisting fruitlets (Adato and Gazit, 1977; Garner and Lovatt, 2016).

Despite the contribution of the above studies to our understanding of avocado IFA, it is yet to be resolved how fruitlets transition to abscission and whether growth arrest and seed coat senescence precede detachment, and on what timescale. Furthermore, an understanding of the early hormone signaling and developmental reprogramming that influence the fate of the seed coat to trigger abscission is lacking. To address these questions, we examined the potential role of the seed coat in avocado IFA. Specifically, we tested the hypothesis that hormone reprogramming in the seed coat initiates a senescence program of development that arrests growth and commits the fruitlet to abscission.

## Materials and methods

### Plant material

Trials were conducted at well-managed orchards on 3–4-year-old trees of the *Persea americana* cultivar ‘Hass’. The fruitlet abscission trial using digital dendrometers to evaluate whether changes in growth rate preceded

abscission was performed at an orchard in the Riverland region of South Australia (34.070312 S, 140.818789 E). The two trials used to harvest fruitlets for transcriptome and hormone analyses together with the gene-expression validation trial were performed in south-west Western Australia (33.63557 S, 115.46191 E). For quantifying the senescence phenotype in the seed coat, immature fruit were harvested from trees in an orchard in central Queensland (25.05442 S, 152.24477 E).

### Calculating growth rate using digital dendrometers

Digital dendrometers (Phytech, Rosh Haayin, Israel) were used to measure daily changes in the diameter of randomly tagged fruitlets from 73–129 days after full bloom (DAFB) in the Riverland orchard. At 73 DAFB, the mean diameter of the tagged fruitlets was 36.5 mm. The dendrometers recorded the fruit diameter every hour, and the change over 24 h represented the daily growth rate ( $\text{mm d}^{-1}$ ). Cumulative abscission was determined by recording the percentage of fruitlets that abscised per tree over the course of the trial.

### Experimental design for examining normal- and arresting-growth fruitlets

Two trials were established at the orchard in Western Australia to identify the developmental and hormonal signaling events associated with fruitlet growth arrest.

#### Natural abscission.

In this trial, a total of 192 fruitlets were randomly tagged across six trees and the diameter of each was measured at regular time intervals using Kincome K11105 Digital Callipers (Fig. 1A) and the mean growth rate was determined ( $\text{mm d}^{-1}$ ). When the mean diameter was 43.8 mm, six fruitlets maintaining a normal pattern of growth, natural abscission-normal (NA-Normal) and six fruitlets showing arresting growth (NA-Arresting) were harvested. The mean growth rates for the NA-Normal and NA-arresting samples were  $0.49 \text{ mm d}^{-1}$  and  $0.03 \text{ mm d}^{-1}$ , respectively, whilst the mean diameters were 42.6 mm and 41.6 mm, respectively. After harvesting, the embryo, seed coat, and pericarp tissues, these samples were frozen on dry ice for subsequent RNA-sequencing and hormone quantification. The latter was carried out on all six fruitlets, whilst five were used for RNA-seq. As fruitlets were the experimental unit for our trials, each one was treated as a biological replicate.

#### Abscission induced by defoliation.

In this trial, fruitlets were captured at an early and a late stage of growth arrest. A total of 100 fruitlets were randomly tagged across three trees, which were manually defoliated by removing the vegetative flush apical to the fruit (Fig. 1B). In parallel, 80 fruitlets were randomly tagged in three untreated control trees. At the start of the trial, the mean diameter of the developing fruitlets across all six trees was 36 mm. In defoliated trees, fruitlet abscission was induced between 8 d and 15 d after treatment, and ultimately resulted in ~97% abscission (Supplementary Fig. S1). Kincome K11105 digital callipers were used to measure the diameter of the tagged fruitlets at 0, 4, 6, and 12 d after defoliation, and the growth rate was calculated ( $\text{mm d}^{-1}$ ). At 6 d after defoliation, five fruitlets showing a normal pattern of growth were harvested from the untreated-control trees (UtC-Normal) and from the defoliated trees, which were in the early stage of growth arrest (DEF-Early); the mean growth rates were  $0.65 \text{ mm d}^{-1}$  and  $0.60 \text{ mm d}^{-1}$ , respectively. On the same day, five fruitlets that were in the late stage of growth arrest (DEF-Late;  $0.10 \text{ mm d}^{-1}$ ) were harvested. The mean diameters for UtC-Normal, DEF-Early, and DEF-Late sampled fruitlets were 39.4, 38.7, and 38.3 mm, respectively. Embryos, seed coats, and pericarps were separated

and frozen on dry ice for subsequent RNA-seq. Again, each fruitlet was treated as a biological replicate.

### Extraction of RNA from fruit tissues

Total RNA was extracted from the seed coat, embryo, and pericarp ( $n=5$  for each tissue) using a Spectrum Plant Total RNA kit (Sigma-Aldrich, #STRN250) following the manufacturer's instructions. RNA was eluted once in 50  $\mu\text{l}$  of the elution buffer supplied with the kit, and residual DNA was removed using a TURBO DNA-free Kit (ThermoFisher Scientific, #AM1907) in a total volume of 60  $\mu\text{l}$ . Library preparation and sequencing were performed by the RNA sequencing service of the Australian Genome Research Facility Ltd (AGRF, Melbourne, Australia). Libraries were sequenced on an Illumina NovaSeq 6000 system, generating 150 bp paired-end (PE) reads.

### Transcriptomic analysis

RNA-seq libraries were prepared from the seed coat, embryo, and pericarp RNA samples derived from the natural and defoliation fruit abscission trials (Fig. 1A, B). Five RNA samples per tissue type were used to prepare the libraries. Across both trials, ~3.15 billion PE raw reads were obtained (~42 million reads per library). Read quality was assessed with FastQC v0.11 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Adapters and low-quality bases were trimmed using Trim Galore v0.6.6 (paired-end mode) (<https://www.trimgalore.com/>). Trimmed reads were aligned to the *Persea americana* cv. 'Hass' genome (Rendón-Anaya *et al.*, 2019) using STAR v2.7 (Dobin *et al.*, 2013), and transcripts were assembled with Cufflinks v2.2.1 (Trapnell *et al.*, 2010). Gene-level read counts were generated with HTSeq (Putri *et al.*, 2022). Transcripts were annotated by BLASTX (Altschup *et al.*, 1990), using NCBI-blast<sup>8</sup> v2.10.1+ against the non-redundant protein sequence database and the Swiss-Prot protein sequence database in BLASTDB v5. Additional BLASTX runs were restricted to *Arabidopsis thaliana* (TaxID: 3702). Annotations of the 25 211 predicted genes were assigned using the top hit with E-value  $\leq 1 \times 10^{-3}$ , prioritizing Swiss-Prot accessions and Arabidopsis gene descriptions where available.

Differential gene expression analysis was performed using the Bioconductor R (v. 4.0.2) package DESeq2 v.1.28.1 (Love *et al.*, 2014). Counts were normalized and differentially expressed genes (DEGs) were identified using the likelihood ratio test in DESeq2 with adjusted  $P \leq 0.05$  and  $\log_2(\text{fold-change}) \geq 1$ . Variance stabilizing transformation was applied before visualization. To identify candidate genes associated with fruitlet growth arrest in the seed coat, pericarp, and embryo, gene ontology (GO) analysis followed by Venn diagram analysis were used to define DEGs shared between the different conditions.

### Quantitative real-time PCR

To validate the RNA-seq results, a separate trial at the Western Australia orchard was established, in which the growth rate of 73 fruitlets was evaluated across three trees using the Kincome K11105 Digital callipers. When the cohort mean diameter was 39.5 mm, six persisting and six arresting fruitlets were harvested (Supplementary Fig. S2). The mean diameters of the six normal-growing and six arresting fruitlets were 39.6 mm and 38.3 mm, respectively, and their growth rates were  $0.56 \text{ mm d}^{-1}$  and  $0.12 \text{ mm d}^{-1}$ , respectively. The seed coats were isolated and pooled into pairs to generate three biological replicates per condition. Total RNA extraction followed the protocol as described above. A QuantiNova Reverse Transcription Kit (Qiagen) was used to synthesize cDNA according to the manufacturer's instructions. Real-time quantitative (qRT)-PCR was performed using the Rotor-Gene Q 5plex platform with the QuantiNova SYBR Green PCR Master Mix according to the manufacturer's instructions (both Qiagen). *AGD12\_1-like* and *ASIL2\_1-like* were selected as reference genes for qRT-PCR analysis

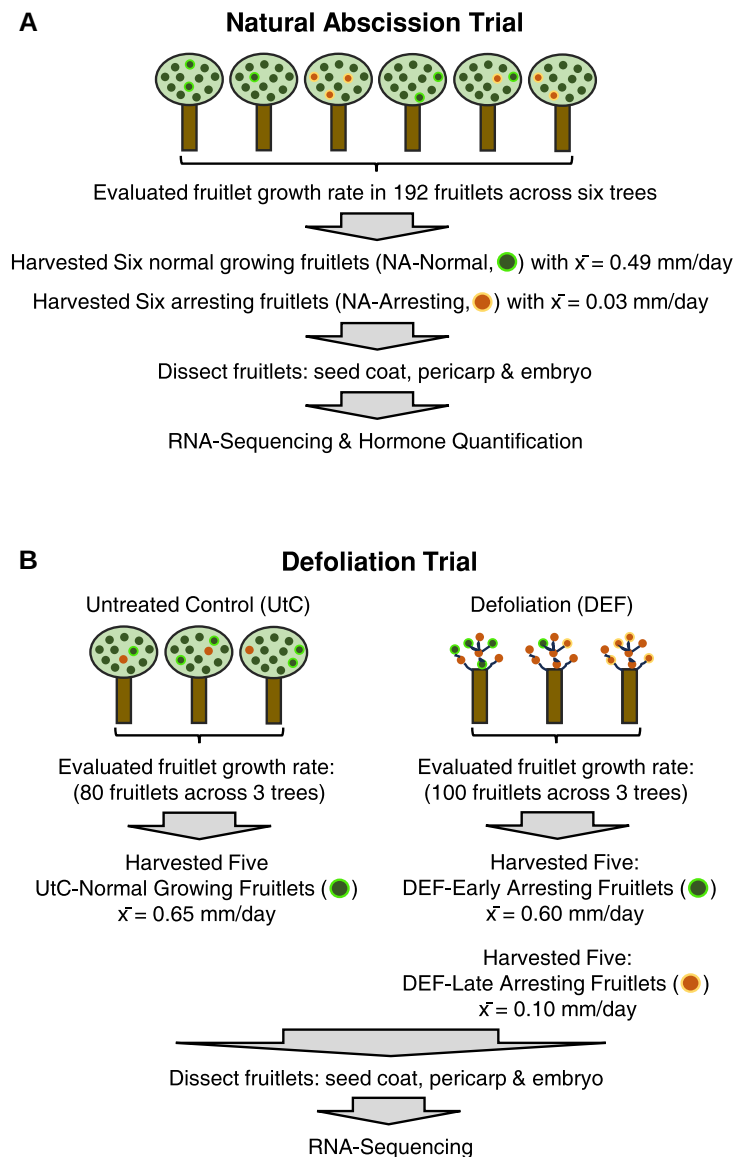


**Fig. 1.** Experimental design for capturing persisting and abscising avocado fruitlets undergoing growth arrest. (A) Natural abscission trial. The diameter of 192 fruitlets was monitored at regular intervals across six trees, and six normal-growing (NA-Normal) and six arresting (NA-Arresting) fruitlets with different mean growth rates were harvested. The seed coat, pericarp, and embryo were used for RNA-sequencing and hormone profiling. (B) Defoliation-induced abscission trial. The growth rate of 100 fruitlets across three defoliated trees and 80 fruitlets across three untreated control trees were evaluated at regular intervals. At 6 d after defoliation, five normal-growing (DEF-Early) and five arresting (DEF-Late) fruitlets with mean growth rates of 0.60 mm d<sup>-1</sup> and 0.10 mm d<sup>-1</sup>, respectively, were harvested from the defoliated trees. On the same day, normal-growing fruitlets with a mean growth rate of 0.65 mm d<sup>-1</sup> were also harvested from untreated control trees (UtC-Control). The seed coats, pericarps, and embryos were used for RNA-sequencing. The normal-growing and arresting fruits are shown schematically as green and orange dots, respectively; the light-green and light-orange outlines show how the harvested fruitlets were distributed across the trees used in the experiments.

because their expression levels remained stable across the persisting and arresting transcriptome datasets. Relative expression levels were calculated using the  $2^{-\Delta\Delta CT}$  method for validation (Livak and Schmittgen, 2001; Supplementary Fig. S3). The primers used for qRT-PCR are given in Supplementary Table S1.

#### Quantification of hormones and related metabolites

For quantification of indole-3-acetic acid (IAA) and IAA conjugated with aspartic acid (IAA-Asp), 50 mg of the seed coat, pericarp, and embryo

samples dissected from NA-Normal and NA-Arresting fruitlets in the natural abscission trial (Fig. 1A) were ground to a fine powder in liquid nitrogen using a Cryomill (Retsch®, Haan, Germany). Six biological replicates were used for each tissue and condition, except for the seed coat of arresting fruitlets where five biological replicates were used. Extraction was carried out in 1.0 ml of ice-cold 50% (v/v) aqueous acetonitrile containing 500 pmol of deuterated IAA-d5 (Cambridge Isotope Laboratories, Andover, USA) and IAA-Asp-d5 (Böttcher *et al.*, 2010) as previously described (Clayton-Cuch *et al.*, 2021). Briefly, after vortexing and sonicating the samples at 4 °C, the samples were centrifuged at 14

000 g and the supernatants were loaded onto a HLB-SPE cartridge (30 mg, Waters) pre-conditioned with methanol, nanopore water, and 50% (v/v) acetonitrile. After collecting the flow-through, the cartridge was rinsed with 1.0 ml 30% (v/v) acetonitrile and the flow-through and eluate were combined and dried at 50 °C in a vacuum concentrator (SP Genevac miVac, Ipswich, UK). The samples were resuspended in 50 µl of 30% (v/v) acetonitrile. IAA and IAA-Asp were analysed by LC coupled with tandem MS (LC-MS/MS) as previously described (Böttcher *et al.*, 2010; Clayton-Cuch *et al.*, 2021). Briefly, samples were analysed on an Agilent 1260 Infinity II HPLC with an Agilent 6470 Triple Quad MS equipped with a jet stream ionization source. A Luna C18 column (75×4.6 mm, 5 µm; Phenomenex, Torrance, CA, USA) was used to separate metabolites after injecting 10 µl of each sample. IAA and IAA-Asp were eluted with a gradient of 0.01% formic acid in water and 0.01% formic acid in 90% (v/v) acetonitrile with a flow rate of 0.35 ml min<sup>-1</sup>. Multiple reaction monitoring-MS was used to detect IAA and IAA-Asp.

In a separate extraction, ABA, phaseic acid (PA), dihydrophaseic acid (DPA), ABA glucose ester (ABA-GE), jasmonic acid (JA), and (+)-7-iso-jasmonoyl-L-isoleucine (JA-Ile) were quantified from 50 mg of each tissue (same samples and replication as above; Fig. 1A). The extraction followed the protocol described above with internal standards added as follows: 5 ng of d6-ABA, d3-PA, d3-DPA and d5-ABA-GE (National Research Council Canada, Saskatchewan, Canada) plus 250 pmol of d5-JA and 25 pmol of d2-JA-Ile (OlChemIm Ltd., Olomouc, Czech Republic).

For quantification of 1-aminocyclopropane-1-carboxylic acid (ACC), a third extraction (50 mg tissue, same samples and replication as above) was performed as described above with ACC-d4 added as internal standard (OlChemIm Ltd). Using the same LC-MS/MS system, ACC was quantified by injecting 2 µl of the resulting extract onto a HILIC-Z column (Poroshell 120 HILIC-Z 2.1×100 mm, 2.7 µm, Agilent) heated to 30 °C. At 7.4 min with a flow rate of 0.2 ml min<sup>-1</sup>, ACC and ACC-d4 were eluted with 20 mM ammonium formate in water (eluent A) and a gradient of 20 mM ammonium formate in 90% (v/v) acetonitrile (eluent B): 100–30% B (0–10 min), 30% B (10–15 min), and 100% B (15–20 min).

#### Quantification of the senescence phenotype in the seed coat during growth arrest

A population of fruitlets ( $n=127$ ) approximately 51 mm in diameter were randomly fitted with digital dendrometers (Phytech) across 14 trees at the Queensland orchard to monitor fruitlet growth. To characterize the timing of seed coat senescence, fruitlets with growth rates ranging from  $-1.15$  mm d<sup>-1</sup> to  $0.74$  mm d<sup>-1</sup> were sampled. These fruitlets were further categorized based on their pattern of growth during the 10 d prior to harvest. After sampling, each fruitlet was cut in half, the embryo removed, and both halves of the seed coat were imaged together at a fixed distance. The senescence phenotype was quantified via image analysis using the Fiji image processing software (v1.53c; Schindelin *et al.*, 2012). Briefly, the polygon tool was used to accurately outline and select each seed-coat half before a consistent colour threshold was applied with minimum and maximum values for hue (5–101), saturation (101–255), and brightness (150–255) to isolate the area of senescence. The percentage area of senescence for each seed-coat half in pixels was determined, and the overall percentage was then calculated for each whole fruitlet.

#### Statistical analysis

Student's *t*-tests were used for pairwise comparisons and one-way ANOVA followed by Tukey's honestly significant difference (HSD) test were performed for multiple comparisons. All analyses were carried out in GraphPad Prism v10.4.1.

## Results

### Fruitlets undergo growth arrest prior to abscission

Digital dendrometers were utilized to characterize the daily growth patterns of developing fruitlets starting from 73 DAFB, when the mean fruitlet diameter was 36.5 mm (Fig. 2A). Over the course of the trial, the mean diameter of developing fruitlets that persisted increased at a steady rate. At the beginning of the trial (73–79 DAFB), these fruitlets had a mean growth rate of  $0.57$  mm d<sup>-1</sup>, which declined to  $0.32$  mm d<sup>-1</sup> by the end (123–129 DAFB; Fig. 2B), at which point they had a mean diameter of 62.3 mm. Fruitlet abscission occurred continuously, with trees dropping a mean of 47.4% of fruitlets by the end of the trial (Fig. 2C). The peak of abscission coincided with a mean fruitlet diameter of 46.6 mm (Fig. 2D). The growth rate of fruitlets that abscised over the course of this trial was collated and plotted against days to fruit drop (Fig. 2E), which showed that fruitlets fated to abscise underwent growth arrest before they shrunk and abscised from the tree. The slowing and eventual cessation of fruitlet growth took ~6 d, followed by another 4–6 d for the shrinking fruitlet to abscise.

### The transcriptome of maternal organs is highly responsive to fruitlet growth arrest

To gain insights into the developmental basis of fruitlet growth arrest under natural conditions, transcriptomes were generated for the seed coat, pericarp, and embryo samples derived from five actively growing (NA-Normal) fruitlets and five immature fruitlets sampled at a late stage of growth arrest (NA-Arresting) (Fig. 1A). In a separate trial, defoliation was used to induce immature fruit abscission (IFA), which caused a mean of 97% of the fruitlets to abscise (Supplementary Fig. S1). As fruitlets underwent growth arrest prior to abscission after defoliation, we used this inducible system to capture fruitlets at an early and late stage of growth arrest. Specifically, five immature fruitlets that maintained a normal growth rate but were expected to undergo subsequent growth arrest were sampled 6 d after defoliation to identify the transcriptome associated with early growth arrest (DEF-Early; Fig. 1B). At the same time-point, five fruitlets that already showed a switch from a normal to a low growth rate were also sampled to identify the transcriptome associated with late growth arrest (DEF-Late). In parallel, five normal-growing fruitlets were sampled from untreated control trees (UtC-Normal) at the same time.

Principal component analysis for the seed coat, pericarp and embryo transcriptomes derived from the natural abscission (NA-Normal and NA-Arresting) and defoliation-induced abscission (DEF-Early, DEF-Late and UtC-Normal) trials showed that normal-growing (NA-Normal and UtC-Normal) and arresting (NA-Arresting, DEF-Early, and DEF-Late) tissue samples were primarily separated by the first two principal components, which explained 52% and 23% of the variance in the dataset, respectively (Fig. 3A). The variance between the normal-growing

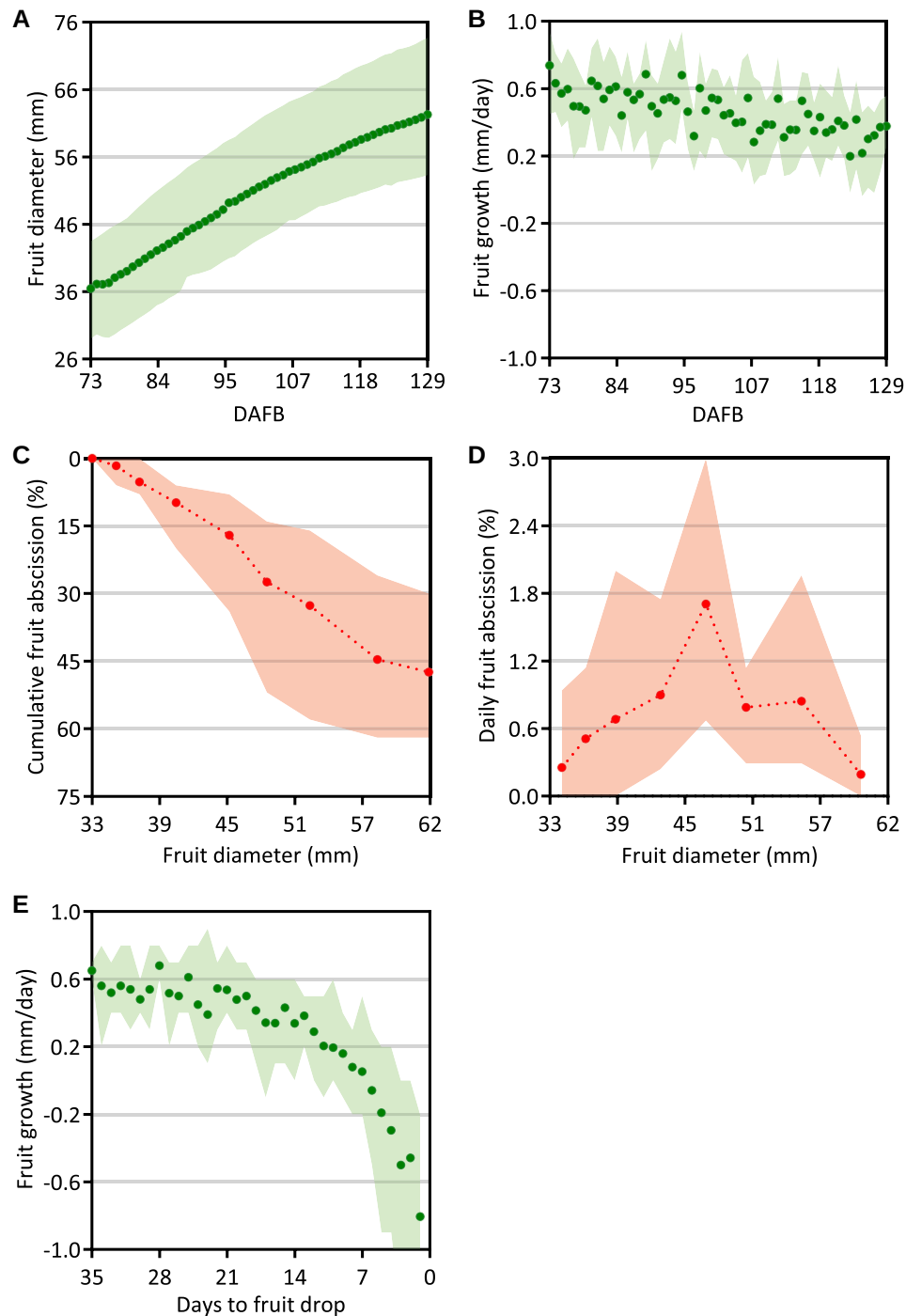


**Fig. 2.** Patterns of growth arrest during immature fruit abscission in avocado. (A) Daily growth of fruitlets that persisted through the summer fruit drop, and (B) their mean growth rate ( $\text{mm d}^{-1}$ ), as determined using dendrometers ( $n=34$ ); DAFB, days after full bloom. (C) Mean cumulative and (D) daily percentage fruitlet abscission during the growing phase of development, expressed on the basis of mean fruitlet diameter ( $n=5$  trees). (E) Mean growth rate of fruitlets that abscised was collated and plotted against days to fruit drop (i.e. abscission occurred on day zero),  $n=22$ . Dots represent means and shaded regions indicate the range of values.

and arresting seed coat and pericarp transcriptomes under natural conditions and in response to defoliation was substantially higher compared to the variance within the embryo transcriptomes. Notably, the transcriptomes for the NA-Arresting and

DEF-Late samples, which captured fruitlets at a late stage of growth arrest, clustered together. The principal component analysis also showed that the maternal transcriptomes of DEF-Early clustered along an axis that was more closely positioned to the

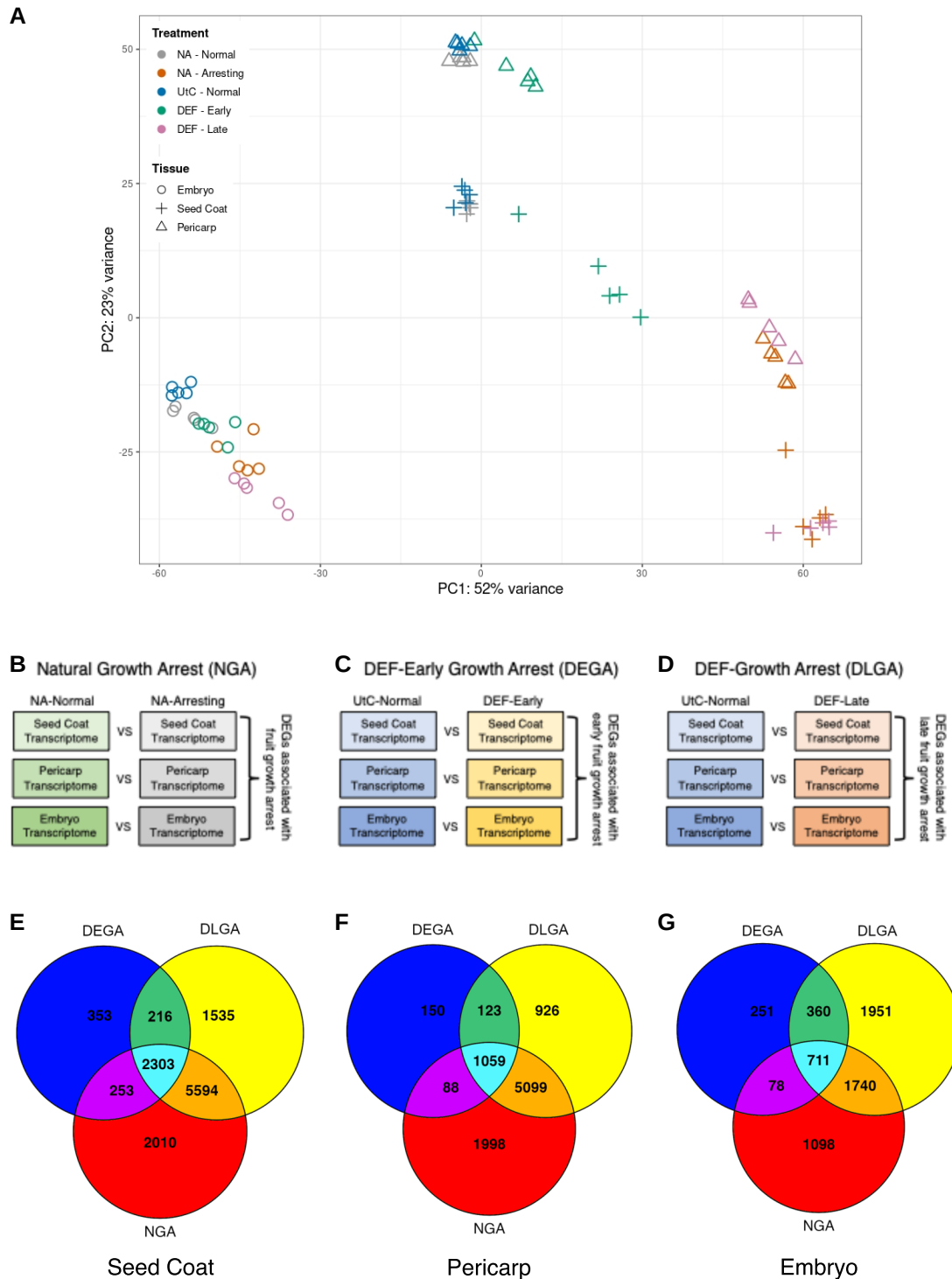


**Fig. 3.** Responses of the seed coat, pericarp, and embryo transcriptomes during fruitlet growth arrest in avocado. (A) Principal component (PC) analysis of the transcriptomes of the different tissues in normal-growing (NA-Normal) and arresting (NA-Arresting) fruitlets under natural conditions, and in fruitlets from defoliated trees showing early and late growth arrest (DEF-Early and DEF-Late, respectively) together with untreated control trees (UtC-Normal). See Fig. 1.  $n=5$ . (B–D) Schematic diagrams of the categories of differentially expressed genes (DEGs) associated with (B) natural growth arrest (NGA), as identified by comparing the transcriptomes of NA-Normal and NA-Arresting for each tissue; (C) defoliation-induced early growth arrest (DEGA), as identified by comparing the transcriptomes of UtC-Normal with DEF-Early; and (D) defoliation-induced late growth arrest (DLGA), as identified by comparing the transcriptomes of UtC-Normal with DEF-Late. (E–G) Venn diagrams showing the numbers of DEGs among the NGA, DEGA, and DLGA comparisons in (E) the seed coat, (F) the pericarp and (G) the embryo.

UtC-Normal than the DEF-Late transcriptomes for the seed coat and pericarp. Furthermore, the DEF-Early seed coat transcriptome displayed a greater degree of variance compared to the DEF-Early pericarp transcriptome. The clustering of the NA-Normal and UtC-Normal seed coat, pericarp, and embryo transcriptomes was expected, as these two sets of fruitlets were harvested only 14 d apart from each other.

To better understand the transcriptional dynamics associated with a growth arrest, differentially expressed genes (DEGs) associated with a late-stage of natural growth arrest (NGA) were identified by comparing the transcriptomes of NA-Normal with NA-Arresting for the three tissue types (Fig. 3B). The temporal changes in gene expression profiles were identified by comparing the tissue transcriptomes obtained from UtC-Normal with DEF-Early and with DEF-Late. By comparing UtC-Normal with DEF-Early, DEGs associated with early growth arrest in response to defoliation were identified (Defoliation-Early Growth Arrest, DEGA; Fig. 3C). Likewise, comparing UtC-Normal with DEF-Late was used to identify DEGs associated with a late stage of growth arrest in response to defoliation (DLGA; Fig. 3D).

Venn diagram analysis was used to identify core sets of DEG implicated in fruitlet growth arrest in each tissue (Fig. 3E-G). There were 2303, 1059, and 711 DEGs in the seed coat, pericarp, and embryo, respectively, that were common to NGA, DLGA, and DEGA. The DEGs shared between DEGA and NGA identified 2556, 1147, and 789 core genes implicated in early growth arrest in the seed coat, pericarp and embryo, respectively. The DEGs shared between NGA and DLGA identified 7897, 6158, and 2451 core genes for the seed coat, pericarp, and embryo, respectively, that were implicated in late growth arrest. As hormones play critical roles in regulating fruit growth and development (Kumar *et al.*, 2014; Fenn and Giovannoni, 2021), as well as senescence (Pautot *et al.*, 2025), we further examined DEGs implicated in auxin, ABA, ET, and JA homeostasis and signaling in the core sets of genes.

#### Fruitlet growth arrest is associated with a reduction in auxin activity

IAA is the major form of auxin that regulates fruit development (Kumar *et al.*, 2014; Fenn and Giovannoni, 2021) and so we investigated its association with growth arrest. IAA production is controlled in part by a two-step pathway, in which tryptophan is first converted to indole-3-pyruvic acid (IPA) via TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA) and TAA-RELATED (TAR; Fig. 4A) (Mashiguchi *et al.*, 2011; Won *et al.*, 2011). In the second step, the rate-limiting YUCCA (YUC) flavin monooxygenase-like enzymes catalyse the conversion of IPA to IAA. In addition to IAA biosynthesis, free IAA is also controlled in part by GRETCHEN HAGEN 3 (GH3) acyl acid amido synthetases, which conjugates IAA with

aspartic acid (Asp), as well as other amino acids, to store, transport, or inactivate it (Staswick *et al.*, 2005).

To investigate a potential role for auxin in fruitlet growth arrest, GO analysis was used to identify *TAA/TAR-like*, *YUC-like*, and *GH3-like* genes with changed expression in response to growth arrest. The results showed that *YUC4-like* and *YUC6-like* genes were down-regulated at late stages of growth arrest in the seed coat and pericarp (Fig. 4B; Supplementary Fig. S4). In addition, their levels of expression were repressed early in the growth-arrest process in the seed coat compared to the pericarp. In contrast, no *TAA/TAR-like* genes were identified as DEGs during growth arrest in the seed coat and pericarp (Supplementary Dataset S1). In response to growth arrest, *GH3.1-like* genes were differentially expressed in the maternal organs (Fig. 4C; Supplementary Fig. S4). *GH3.1-like\_1/3* were up-regulated in the seed coat at both early and late stages of growth arrest, whereas only *GH3.1-like\_1* expression increased in the pericarp at late growth cessation. In addition, *GH3.1-like\_2* and *DFL2-like* were down-regulated in the maternal organs during growth arrest. In the embryo, none of the *TAA/TAR-like* and *YUC-like* genes were identified as DEGs (Supplementary Dataset S1), while *GH3.1-like\_1/3* were up-regulated during late growth arrest (Fig. 4C; Supplementary Fig. S4). Genes that encode auxin transporters and response transcription factors were also differentially expressed, predominantly in maternal tissues and to a lesser extent in the embryo (Supplementary Figs S5, S6).

To further examine a potential role for auxin in IFA, the levels of IAA and IAA-Asp were quantified in the seed coat, pericarp, and embryo of NA-Normal and NA-Arresting fruitlets. The results showed that IAA in the seed coat was 11.4- and 10.2-fold higher than in the pericarp and embryo, respectively, in NA-Normal fruitlets (Fig. 4D). At a late stage of growth arrest, IAA concentrations were significantly reduced in the seed coat and pericarp of NA-Arresting compared to NA-Normal fruitlets. To determine if auxin conjugation was associated with a decrease in free IAA, the concentrations of IAA-Asp were quantified in the different tissues in NA-Normal and NA-Arresting fruitlets. The results showed that IAA-Asp concentration increased by 7.3-fold in the seed coat of NA-Arresting compared to NA-Normal fruitlets (Fig. 4E). While the mean concentration of IAA-Asp also increased in the pericarp in NA-Arresting compared to NA-Normal fruitlets, this was not statistically significant ( $P=0.13$ ). In the embryo, there were no differences in the levels of IAA and IAA-Asp between the NA-Normal and NA-Arresting fruitlets (Fig. 4D, E).

#### Fruitlet growth arrest is associated with accumulation of ABA in the maternal organs

As ABA is implicated in avocado IFA (Adato and Gazit, 1977; Garner and Lovatt, 2016), we investigated whether an increase in its activity in the seed coat, pericarp, and/or embryo was

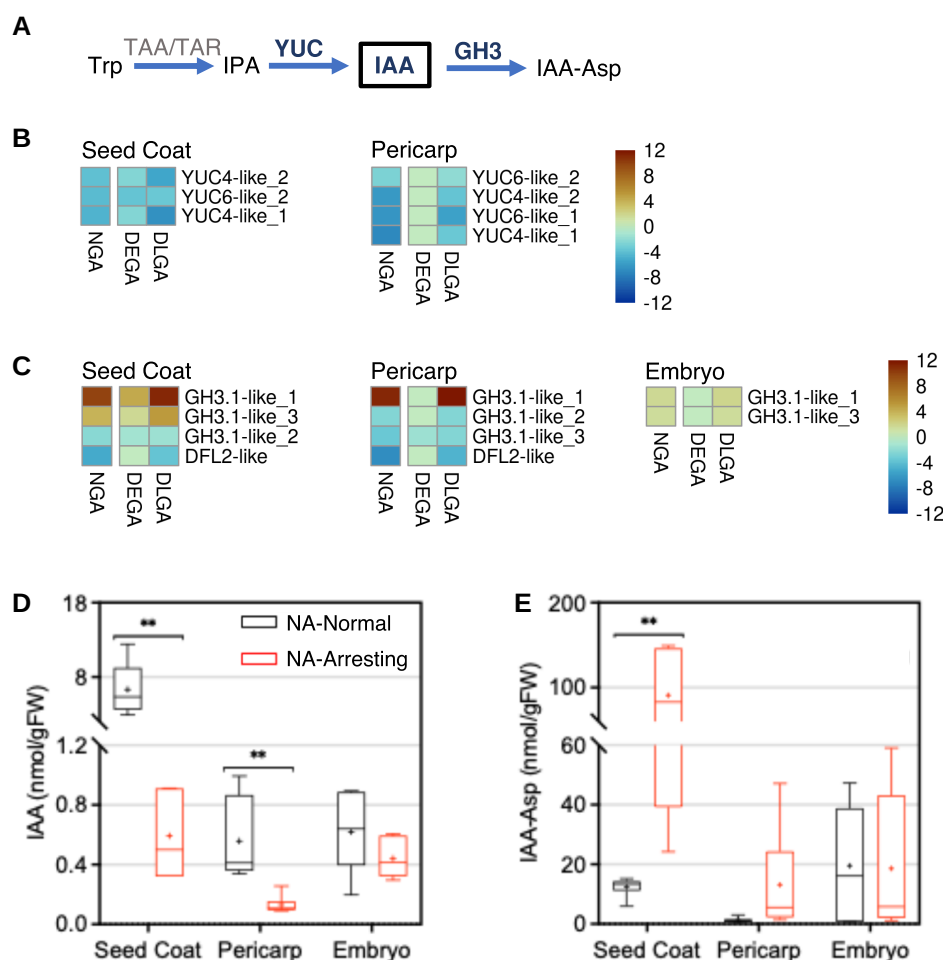


**Fig. 4.** Responses of auxin levels to fruitlet growth arrest in avocado. (A) Schematic diagram of the regulation of IAA by the TAA1/YUC biosynthesis pathway and GH3, which conjugates IAA with aspartic acid (Asp) or other amino acids. (B, C) Heatmaps of differentially expressed auxin-biosynthesis and conjugation genes in the seed coat, pericarp, and embryo of fruitlets undergoing natural growth arrest (NGA), defoliation-induced early growth arrest (DEGA), and defoliation-induced late growth arrest (DLGA), as described in Fig. 3. (B) IAA biosynthesis (*YUCCA*) genes and (C) conjugation (*GH3*) genes. The heatmaps display  $\log_2$ (fold-change) of expression according to the colour gradient. Genes with  $\log_2$ (fold-change)  $\leq 1$  were assigned a value of 0.  $n=5$ . (D) Free IAA and (E) IAA-Asp concentrations in the seed coat, pericarp, and embryo in normal growing (NA-Normal) and arresting (NA-Arresting) fruitlets. Box plots show the interquartile range with maximum and minimum values indicated by whiskers. The mean and median are represented by '+' and a horizontal line, respectively. Six biological replicates were used, except the arresting seed coat where five biological replicates were used. Significant differences were determined using Student's *t*-test: \*\* $P \leq 0.01$ .

associated with growth arrest. ABA is synthesized from the  $C_{40}$ -carotenoid precursors 9-*cis*-neoxanthin and 9-*cis*-violaxanthin in a three-step process mediated by 9-*cis*-epoxycarotenoid dioxygenase (NCED), a short-chain alcohol dehydrogenase (ABA2) and abscisic acid aldehyde oxidase (AAO3; Fig. 5A) (Chen *et al.*, 2020).

GO analysis showed that the expression of *NCED9/3-like* and *AAO3-like* genes in the seed coat increased during both early and late stages of growth arrest, whilst *AAO3-like\_1/2* increased at late growth arrest (Fig. 5B; Supplementary Fig. S7). In the pericarp, ABA biosynthesis genes were differentially expressed, with *NCED9/3-like*, *ABA2-like*, and *AAO3-like* genes up- and/or down-regulated during growth arrest.

*AAO3-like\_1/2* were up-regulated during both early and late stages of growth arrest. In contrast to the maternal organs, the only ABA biosynthesis gene that was differentially expressed in the embryo during growth arrest was *NCED3-like\_2*, which was up-regulated.

Studies in Arabidopsis and maize indicated that the HOMEBOX PROTEIN 40 (HB40)/GRASSY TILLERS1 (GT1) transcription factor inhibits bud outgrowth in part by inducing *NCED* gene expression (Fig. 5A) (Gonzalez-Grandio *et al.*, 2017; Dong *et al.*, 2019). Consistent with this regulatory role, *HB40-like* genes were up-regulated in the seed coat and pericarp at early and late stages of growth arrest in avocado (Fig. 5B; Supplementary Fig. S7). In contrast, no *HB40-like*

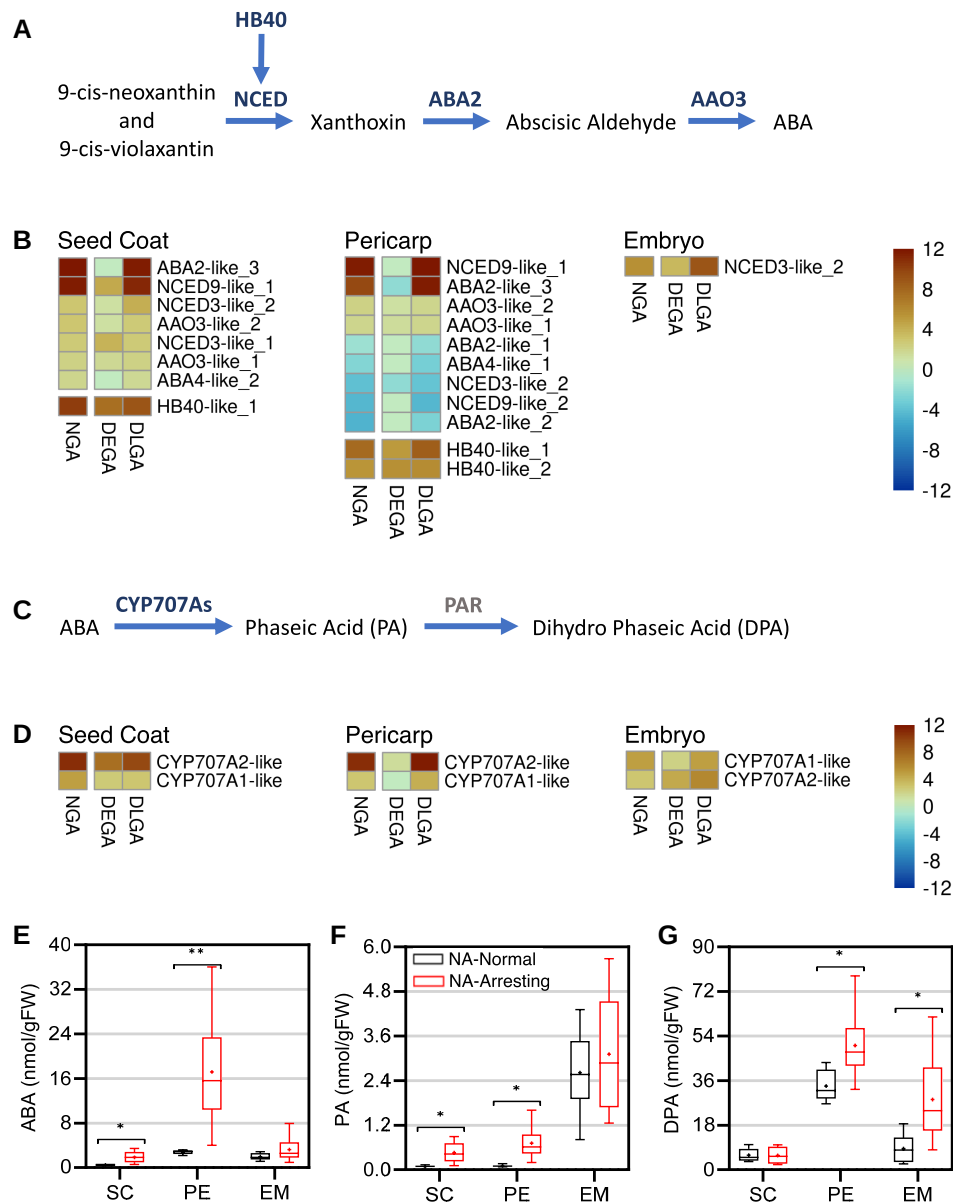


**Fig. 5.** Responses of ABA biosynthesis and catabolism during fruitlet growth arrest in avocado. (A) Schematic diagram of the ABA biosynthesis pathway. NCED catalyses the conversion of 9-*cis*-neoxanthin or 9-*cis*-violaxanthin to xanthoxin, while ABA2 converts xanthoxin to absciscic aldehyde. In the last step, absciscic aldehyde is converted to ABA by AAO3. The homeobox-leucine zipper transcription factor HB40 activates *NCED* gene expression. (B) Heatmaps of differentially expressed ABA biosynthesis genes in the seed coat, pericarp, and embryo of fruitlets undergoing natural growth arrest (NGA), defoliation-induced early growth arrest (DEGA), and defoliation-induced late growth arrest (DLGA), as described in Fig. 3. (C) Schematic diagram of ABA catabolism. ABA is metabolised to phaseic acid (PA) and dihydrophaseic acid (DPA) by CYP707As and PAR, respectively. (D) Differential expression of *CYP707A* genes in the seed coat, pericarp, and embryo during NGA, DEGA, and DLGA. The heatmaps display log<sub>2</sub>(fold-change) of expression according to the colour gradient. Genes with log<sub>2</sub>(fold-change) ≤ 1 were assigned a value of 0. *n* = 5. (E–G) Concentrations of (E) ABA, (F) PA, and (G) DPA in the seed coat (SC), pericarp (PE), and embryo (EM) in normal-growing (NA-Normal) and arresting (NA-Arresting) fruitlets. Box plots show the interquartile range with maximum and minimum values indicated by whiskers. The mean and median are represented by '+' and a horizontal line, respectively. Six biological replicates were used, except the arresting seed coat where five biological replicates were used. Significant differences were determined using Student's *t*-test: \**P* ≤ 0.05, \*\**P* ≤ 0.01.

genes were identified as DEGs in the embryo (Supplementary Dataset S1). ABA catabolism is a two-step process in which ABA is first converted to PA by CYP707A cytochrome P450 monooxygenases (Fig. 5C) (Kushiro et al., 2004; Saito et al., 2004). Subsequently, PHASEIC ACID REDUCTASE (PAR) catalyses PA to DPA (Weng et al., 2016). In the seed coat, CYP707A1/2-like were up-regulated during early and

late stages of growth arrest (Fig. 5D; Supplementary Fig. S8). In contrast, increased expression of *CYP707A1/2-like* in the pericarp occurred only during the late stage of growth arrest. In the embryo, *CYP707A2-like* was induced at both the early and late stages of growth arrest, while *CYP707A1-like* increased only at the late stage of growth arrest. In the GO analysis, no genes encoding a PAR-like enzyme were identified in the DEGs associated with ABA biosynthesis and catabolism (Supplementary Dataset S1).

We next examined the concentrations of ABA, PA, and DPA in the seed coat, pericarp, and embryo isolated from NA-Normal and NA-Arresting fruitlets. In NA-Normal fruitlets, ABA was relatively low in the seed coat compared to the pericarp and embryo (Fig. 5E). At the late stage of growth arrest, ABA increased significantly in the seed coat and pericarp but not in the embryo of NA-Arresting compared to NA-Normal fruitlets. In the maternal organs of NA-Normal fruitlets, the levels of PA were low compared to the embryo (Fig. 5F). In NA-Arresting fruitlets, a significant increase in PA occurred in the seed coat and pericarp compared to NA-Normal fruitlets. DPA concentrations in NA-Normal fruitlets were highest in the pericarp (Fig. 5G) and increased significantly in both the pericarp and embryo in NA-Arresting fruitlets. Conjugation of ABA with glucose to form the ABA-glucose ester (ABA-GE) regulates the activity and localization of this hormone (Chen *et al.*, 2020), but we found that there was no significant change in the levels of ABA-GE in either the seed coat, pericarp, or embryo in NA-Arresting compared to NA-Normal (Supplementary Fig. S9).

### ACC increases in the seed coat during growth arrest

As ET is implicated in avocado IFA (Adato and Gazit, 1977; Davenport and Manners, 1982), we investigated whether its precursor, ACC (used as a proxy for ET), accumulated in the seed coat, pericarp, and/or embryo during growth arrest. ET is produced by a two-step process starting with S-adenosyl-L-methionine (SAM), which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) via ACC synthase (ACS; Fig. 6A) (Pattyn *et al.*, 2021). In the second step, conversion of ACC to ET is mediated by ACC oxidase (ACO). GO analysis of DEGs involved in ET biosynthesis showed that *ACS1-like\_1* and *ACS1-like\_7* were up-regulated during both early and late stages of growth arrest in the seed coat (Fig. 6B; Supplementary Fig. S10). In addition, the expression of *ACS1-like\_2* increased at late growth arrest in the seed coat. In the pericarp and embryo, expression of *ACS1-like\_1* increased during early and late stages of growth arrest. The expression of *ACS7-like* increased during late growth arrest in the pericarp. In contrast to the seed coat and embryo, only the pericarp displayed an up-regulation of an ACO gene, *ACO4-like*, during growth arrest.

We next examined ACC and found that a significant increase in concentration occurred in the seed coat in

NA-Arresting compared to NA-Normal fruitlets (Fig. 6C). On the other hand, despite the up-regulation of *ACS1-like\_1* (Fig. 6B), the concentration of ACC in the embryo was significantly lower in NA-Arresting compared to NA-Normal fruitlets.

### Jasmonate increases in the seed coat during fruit growth arrest

JA negatively regulates growth and promotes senescence (Huang *et al.*, 2017; Guo *et al.*, 2018). The production of JA from alpha-linolenic acid is a multistep process mediated by 13-lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), and 12-oxophytodienoic acid reductase (OPR; Fig. 7A) (Huang *et al.*, 2017). In the final step, JASMONATE RESISTANT 1 (JAR1) conjugates JA with isoleucine (Ile) to produce the bioactive JA-Ile (Staswick and Tiryaki, 2004; Fonseca *et al.*, 2009). GO analysis showed that *LOX4-like*, *LOX5-like\_1*, and *AOS-like\_2/3/4* were up-regulated in the seed coat during both the early and late stages of growth arrest (Fig. 7B; Supplementary Fig. S11). In addition, expression of *LOX2-like\_2* and *LOX5-like\_2/3* also increased in the seed coat during late growth arrest. In contrast, *AOC3-like* and *AOC4-like* were down-regulated during late growth arrest. JA biosynthesis genes were also differentially expressed in the pericarp and embryo but to a lesser extent than the seed coat during growth arrest. Despite the overall up-regulation of *LOX-like*, *AOS-like*, and *AOC-like* in the seed coat, pericarp, and embryo, no *JAR1-like* genes were identified as being differentially expressed during growth arrest (Supplementary Dataset S1).

At the late stage of growth arrest, the concentration of JA significantly increased in the seed coat of NA-Arresting compared to NA-Normal fruitlets, and a modest but significant increase also occurred in the pericarp (Fig. 7C); on the other hand, it remained unchanged in the embryo. The JA-Ile concentrations were extremely low in all the tissues of NA-Normal fruitlets (Fig. 7D), but increased significantly in the seed coat of NA-Arresting fruitlets.

### Seed coat senescence is initiated at late growth arrest

In fruitlets with a mean growth rate  $>0.40 \text{ mm d}^{-1}$ , the seed coat was yellowish in appearance with punctate browning restricted to the vascular connections at the base of this maternal organ (Fig. 8A). In contrast, abscised fruitlets displayed a senescence phenotype in the seed coat (Fig. 8B). To determine the timing of seed-coat senescence in the IFA pathway, we used image analysis to quantify the phenotype (as percentage senescence) of actively growing, arresting, and shrinking fruitlets when the mean diameter of the fruitlets was  $\sim 51 \text{ mm}$  in diameter. The fruitlets were grouped together based on their growth rate at the time of sampling, as well as their overall pattern of growth in the 10 d before harvest (Supplementary Fig. S12).

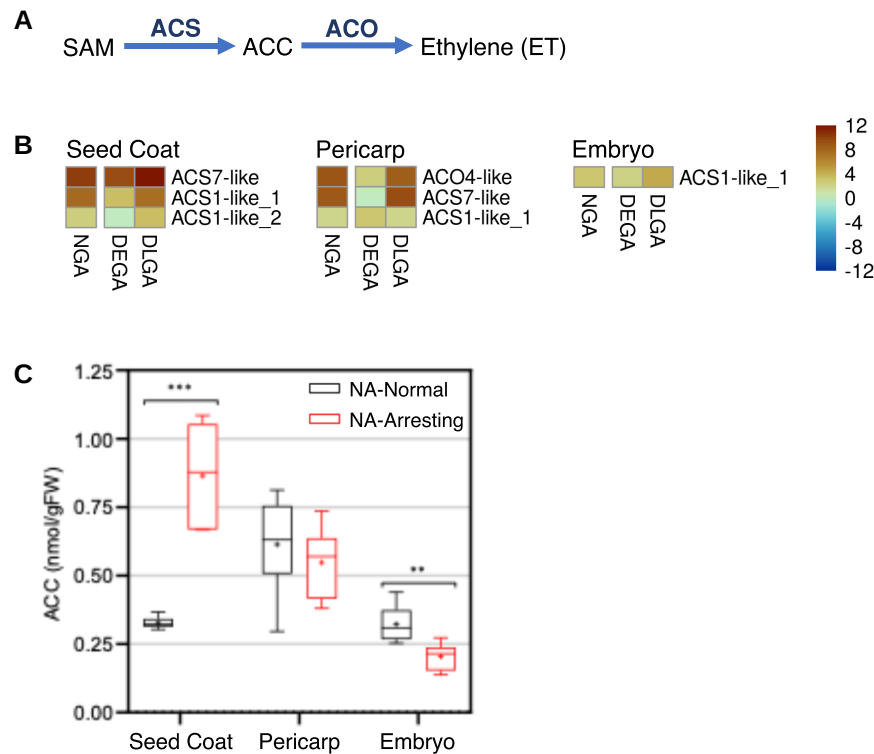


**Fig. 6.** Response of the ethylene (ET) precursor ACC during fruitlet growth arrest in avocado. (A) Schematic diagram of the ET biosynthesis pathway. ACS and ACO catalyse the conversion of SAM to ACC and ACC to ET, respectively. (B) Heatmaps of differentially expressed of ET biosynthesis genes in the seed coat, pericarp, and embryo of fruitlets undergoing natural growth arrest (NGA), defoliation-induced early growth arrest (DEGA), and defoliation-induced late growth arrest (DLGA), as described in Fig. 3.  $n=5$ . The heatmaps display  $\log_2$ (fold-change) of expression according to the colour gradient. Genes with a  $\log_2$ (fold-change) $\leq 1$  were assigned a value of 0. (C) Concentration of ACC in the seed coat, pericarp, and embryo in normal-growing (NA-Normal) and arresting (NA-Arresting) fruitlets. Box plots show the interquartile range with maximum and minimum values indicated by whiskers. The mean and median are represented by '+' and a horizontal line, respectively. Six biological replicates were used, except the arresting seed coat where five biological replicates were used. Significant differences were determined using Student's  $t$ -test: \*\* $P\leq 0.01$ , \*\*\* $P\leq 0.001$ .

We found that the mean percentage senescence in the seed coat of fruitlets that maintained a relatively consistent pattern of growth and a growth rate  $\geq 0.3 \text{ mm d}^{-1}$  was  $<0.7\%$  (Fig. 8C). Fruitlets sampled with a growth rate between  $0.2\text{--}0.29 \text{ mm d}^{-1}$ , which displayed a similar pattern of growth as those at  $\geq 0.3 \text{ mm d}^{-1}$ , also showed a mean percentage senescence  $<0.7\%$  (Fig. 8C; Supplementary Fig. S12). To identify fruitlets that appeared to be committed to abscission, we examined immature fruit with growth rates  $\leq 0.2 \text{ mm d}^{-1}$ , which all displayed a decline in growth for 3 d immediately before sampling. We found that those with a growth rate between  $0.1\text{--}0.19 \text{ mm d}^{-1}$  displayed a minimal mean percentage senescence of  $<1.0\%$  (Fig. 8C). Whilst fruitlets with growth rates of  $0.00\text{--}0.09 \text{ mm d}^{-1}$  had a mean value of  $4.9\%$ , this was not statistically different with those that had a growth rate  $>0.1 \text{ mm d}^{-1}$ . In contrast, fruitlets exhibiting negative growth rates showed significant increases in the seed coat percentage senescence phenotype.

To further characterize the timing of the seed-coat senescence phenotype at the molecular level, GO analysis was

used to identify DEGs that encode positive and negative regulators of senescence. For example, genes such as *AUXIN RESPONSE FACTOR1* (*ARF1-like*), *PHYTOALEXIN DEFICIENT4-like* (*PAD4-like*), *BIFUNCTIONAL NUCLEASE1* (*BFN1*), *SENESCENCE ASSOCIATED GENE 12* (*SAG12*), *ARABIDOPSIS A-FIFTEEN* (*AAF*), and *SMALL AUXIN UP RNA 36* (*SAUR36*), are often used as markers to evaluate the onset and progression of senescence (Noh and Amasino, 1999; Morris et al., 2000; Pérez-Amador et al., 2000; Ellis et al., 2005; Chen et al., 2012; Hou et al., 2013). The results showed that *PAD4-like* and *ARF1-like* were up-regulated during early and late stages of growth arrest in the seed coat (Fig. 8D; Supplementary Fig. S13). However, most senescence-associated genes that positively regulate tissue degeneration were only up-regulated during late growth arrest, including *BFN1-like*, *SAG12-like*, *AAF-like*, and *SAUR36-like*. In addition, transcript abundance for genes homologous to *JUNGBRUNNEN1* (*JUB1*) and *WRKY70*, which are induced during senescence but negatively regulate this process

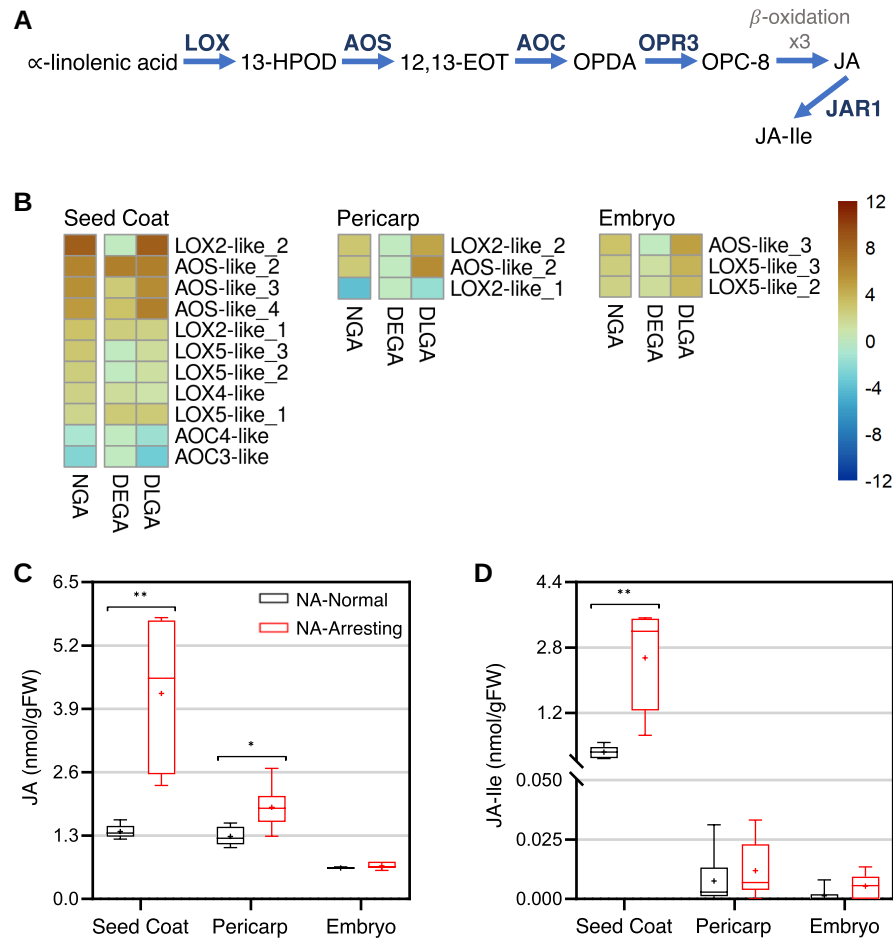


**Fig. 7.** Responses of jasmonates during fruitlet growth arrest in avocado. (A) Schematic diagram of the JA biosynthesis pathway.  $\alpha$ -linolenic acid is converted to JA in a multistep process involving LOX, AOS, AOC, and OPR3. (B) Heatmaps of differentially expressed JA biosynthesis genes in the seed coat, pericarp, and embryo of fruitlets undergoing natural growth arrest (NGA), defoliation-induced early growth arrest (DEGA), and defoliation-induced late growth arrest (DLGA), as described in Fig. 3. The heatmaps show the  $\log_2$ (fold-change) of expression according to the colour gradient. Genes with  $\log_2$ (fold-change)  $\leq 1$  were assigned a value of 0.  $n=5$ . (C) JA and (D) JA-Ile concentrations in the seed coat, pericarp, and embryo of normal-growing (NA-Normal) and arresting (NA-Arresting) fruitlets. Box plots show the interquartile range with maximum and minimum values indicated by whiskers. The mean and median are represented by '+' and a horizontal line, respectively. Six biological replicates were used, except the arresting seed coat where five biological replicates were used. Significant differences were determined using Student's *t*-test: \* $P \leq 0.05$ , \*\* $P \leq 0.01$ .

(Besseau *et al.*, 2012; Wu *et al.*, 2012), increased during growth arrest. A subset of senescence-associated genes was also up-regulated in the pericarp and embryo during late growth arrest (Supplementary Dataset S1). However, in contrast to the seed coat, the pericarp and embryo displayed little or no visible signs of senescence (Fig. 8B). In addition to senescence-associated genes, ET- and JA-signaling genes implicated in senescence were also differentially expressed during fruitlet growth arrest (Supplementary Figs S14, S15).

Late growth arrest is associated with transcriptional changes implicated in seed dormancy

In persisting and recently abscised avocado fruitlets the embryo displayed no visible signs of tissue degeneration, whereas the

seed coat of abscised fruitlets displayed visible signs of senescence (Fig. 9A, B). Given the apparent absence of tissue degeneration in the embryo, we speculated that the seed might have undergone a dormancy-like program of development during growth arrest. As seed dormancy is positively regulated in part by ABA signaling derived from maternal organs and zygotic tissues (Chen *et al.*, 2020 Iwasaki *et al.*, 2022), we used GO analysis to identify DEGs involved in ABA and dormancy-associated signaling pathways. We found that at least one member of *PYRABACTIN RESISTANCE* (*PYR*)/*PYR-LIKE* (*PYL*), encoding an ABA receptor, was induced in response to growth cessation in the seed coat, pericarp, and embryo (Fig. 9D; Supplementary Fig. S16). For example, *PYL9-like\_2*, *PYL8-like*, and *PYL4-like\_1* were up-regulated during early and late stages of growth arrest in the seed coat.

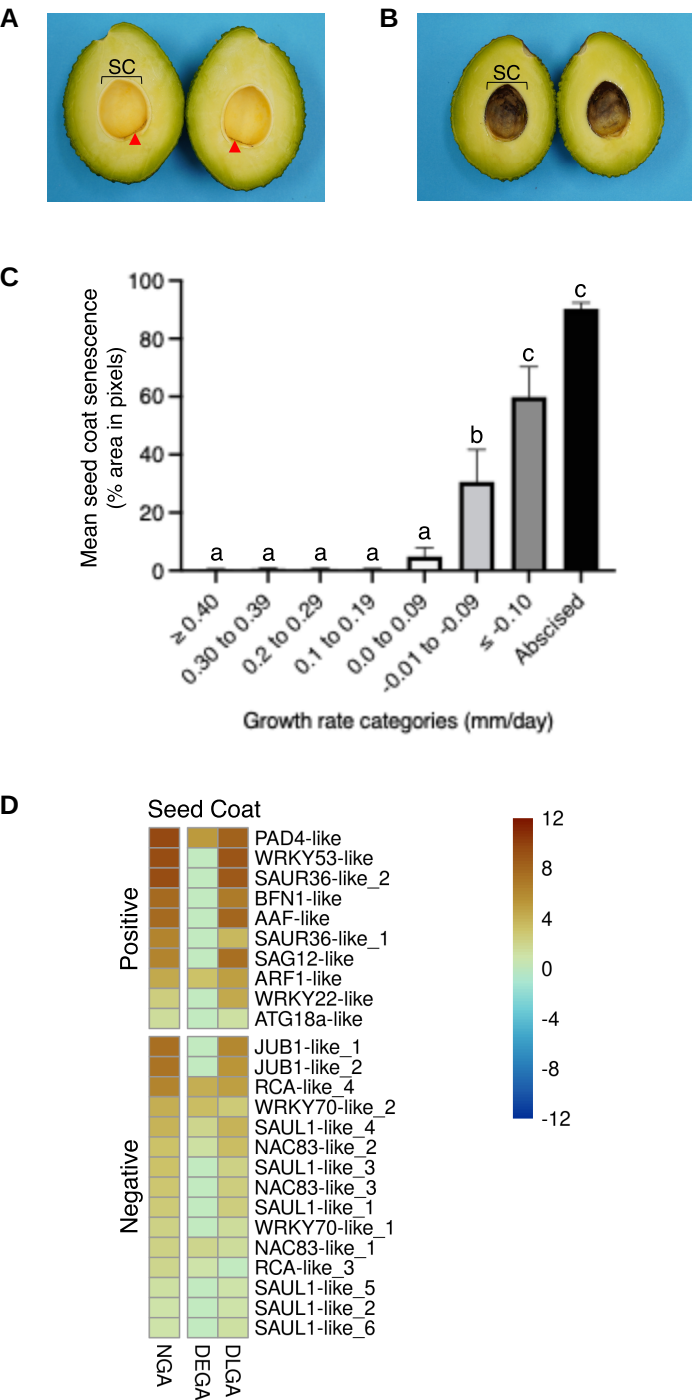


**Fig. 8.** Timing of seed-coat senescence in the immature fruit abscission pathway in avocado. (A, B) Representative images of the seed coat (SC) in (A) normal-growing and (B) recently abscised fruitlets. The seed coat was visualized by removing the embryo. (C) The senescence phenotype of the seed coat was quantified by analysis of images taken from a total of 127 fruitlets exhibiting different growth rates, as shown in [Supplementary Fig. S12](#). The mean percentage of senescence-associated pixels per seed coat image is plotted for each growth-rate category. Data are means ( $\pm$ SEM),  $n=13$ –25 except  $n=6$  for abscised fruitlets. Different letters indicate significant differences among means as determined using one-way ANOVA

In the embryo, only *PYL4-like\_1* was also up-regulated during early and late stages of growth arrest. The expression of at least one gene that encoded a SUCROSE NONFERMENTING-1-RELATED KINASE 2.6 (*SnRK2.6*), which positively regulates ABA signaling ([Chen et al., 2020](#)), increased in the seed coat, pericarp, and embryo during growth arrest. For example, in the seed coat and embryo, *SnRK2.6-like\_5* was up-regulated during late growth arrest, *SnRK2.6-like\_2* was up-regulated during early and late stages of growth arrest, while *SnRK2.6-like\_6* was only up-regulated at late growth arrest in the seed coat. In the maternal organs, growth arrest was also associated with the up-regulation of *ABA INSENSITIVE 1-like\_1* (*ABI1-like\_1*) and *ABA-RESPONSIVE ELEMENT BINDING FACTOR2-like* (*ABF2-like*). In contrast to *ABI1-like\_1*, *ABI3-like* was down-regulated in the pericarp during growth arrest. In the embryo, no *ABI-like* or *ABF2-like* genes were identified by GO analysis as being differentially expressed during growth arrest ([Supplementary Dataset S1](#)).

In addition to ABA signaling, the dormancy potential of a seed is also influenced by FLOWERING LOCUS T (FT) in the seed coat, which acts to delay dormancy ([Fig. 9C](#)) ([Chen et al., 2014](#)). In contrast, MOTHER OF FT AND TFL1 (MFT) acts in the embryo to promote dormancy, in part by modulating ABA signaling ([Fig. 9C](#)) ([Xi et al., 2010](#); [Nakamura et al., 2011](#); [Footitt et al., 2017](#)). We found that the expression of *FT-like\_1/2* were down-regulated during growth arrest in the seed coat and pericarp ([Fig. 9E](#); [Supplementary Fig. S17](#)). In these maternal organs, *MFT-like\_2* was also suppressed during growth arrest. However, *MFT-like\_3* was up-regulated at late growth arrest in the seed coat. In contrast to the seed coat and pericarp, *MFT-like\_1/2* were up-regulated in the embryo during late growth arrest.

## Discussion

### Origin of the immature fruit abscission signal

The seed coat plays a critical role in regulating seed and fruit development ([Radchuk and Borisjuk, 2014](#); [Figueiredo and Köhler, 2018](#); [Robert, 2019](#)). For example, studies have indicated that signals derived from the seed coat regulate embryo patterning, seed maturation, and dormancy ([Radchuk and Borisjuk, 2014](#); [Robert et al., 2018](#); [Iwasaki et al., 2022](#)). Based on the senescence phenotype displayed in the seed coat, we hypothesized that this maternal organ plays a critical role in immature fruit abscission (IFA) in avocado.

**Fig. 8.** Continued followed by Tukey's HSD test (adjusted  $P<0.05$ ). (D) Heatmaps of differentially expressed senescence-associated genes in the seed coat of fruitlets during natural growth arrest (NGA), defoliation-induced early growth arrest (DEGA), and defoliation-induced late growth arrest (DLGA). The heatmaps show the  $\log_2$ (fold-change) of expression displayed according to the colour gradient. Genes with a  $\log_2$ (fold-change) $\leq 1$  were assigned a value of 0.  $n=5$ .

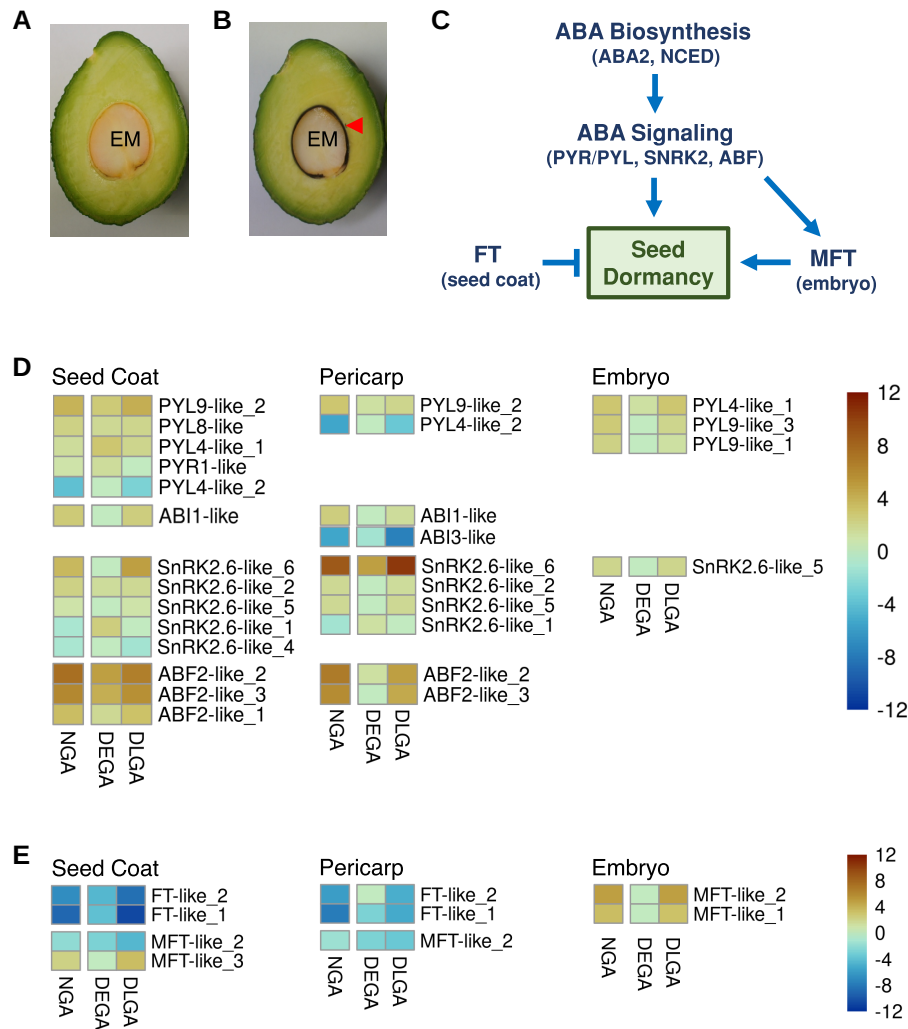


**Fig. 9.** Differential expression of seed-dormancy signaling genes during fruitlet growth arrest in avocado. (A, B) Representative images of (A) a persisting and (B) a recently abscised fruitlet; EM, embryo; the red arrowhead indicates the seed coat. (C) Schematic diagram of dormancy signaling pathways. (D) Heatmaps of differentially expressed ABA-associated dormancy-signaling genes in the seed coat, pericarp, and embryo during natural growth arrest (NGA), defoliation-induced early growth arrest (DEGA), and defoliation-induced late growth arrest (DLGA), as described in Fig. 3. (E) Heatmaps of differential expression of FT- and MFT-associated genes. The heatmaps show the  $\log_2(\text{fold-change})$  of expression according to the colour gradient. Genes with a  $\log_2(\text{fold-change}) \leq 1$  were assigned a value of 0.  $n=5$ .

Consistent with this hypothesis, transcriptome reprogramming induced during fruitlet growth arrest was substantially greater in the seed coat and pericarp compared with the embryo (Fig. 3). Further, during early growth arrest, the seed coat exhibited a greater number of DEGs than the pericarp and embryo. Together, these results indicate that the seed coat is the maternal organ that perceives and transmits the IFA signal(s) to the pericarp and embryo to coordinate developmental programs required for abscission. It is highly conceivable that the IFA signaling event(s) transmitted from the seed coat to induce growth arrest is mediated in part by a signal(s) that mediates the transition from growth to senescence, as well as the activation of a dormancy-like pathway in the seed.

If the seed coat is the site where IFA signaling is initiated, what is the nature of the signal(s)? Previous studies have indicated that auxin increases in the seed coat upon fertilization, where it regulates embryo and seed development (Robert *et al.*, 2018; Robert, 2019). As seed-derived auxin is thought to regulate fruit development (Robert, 2019; Fenn and Giovannoni, 2021), its movement from the seed coat to the pericarp might also act to coordinate growth of the fruit tissues. Further, a reduction of auxin activity is critical for organs to acquire the competence to undergo senescence and abscission (McAtee *et al.*, 2013; Mueller-Roeber and Balazadeh, 2014; Patharkar and Walker, 2019; Fenn and Giovannoni, 2021). Our results indicated that the seed coat is the primary site of

IAA production in developing avocado fruitlets during summer IFA event, with concentrations being 10- to 11-fold higher in the seed coat than in the pericarp and embryo, respectively (Fig. 4). Furthermore, at the late stage of fruitlet growth arrest, free IAA concentrations were significantly reduced in the seed coat. The seed coat also exhibited changes in expression of auxin biosynthesis (*YUC4/6-like*) and IAA-conjugation (*GH3.1-like\_1*) genes earlier in the growth arrest process than the pericarp. Lastly, the decrease in free IAA in the seed coat during growth arrest might ultimately reduce its export and polar transport through the pedicel, thereby allowing ET to promote cell separation and detachment. Taken together, we hypothesize that the reduction in seed-coat auxin levels is the primary signaling event that is transmitted to the pericarp and embryo to induce growth cessation and commit the arrested fruitlet to abscission.

Growth arrest is an initial step in the avocado fruitlet abscission pathway

Studies in avocado and apple indicate that a slowing of fruitlet growth precedes abscission (Ward and Marini, 1999; Greene *et al.*, 2013; Garner and Lovatt, 2016). Under both natural conditions and in response to defoliation, our results showed that fruitlets fated to abscise underwent growth arrest before shrinking and detaching from the tree (Fig. 2; Supplementary Fig. S1). The manifestation of the growth arrest phenotype early in the avocado IFA pathway was further supported by the fact that a substantial number of genes that regulate growth were differentially expressed in the seed coat, pericarp, and embryo (Supplementary Dataset S1), including the overall down-regulation of genes that regulate progression of the cell cycle (Supplementary Fig. S18). Therefore, we hypothesize that the previously proposed feedback interactions between expanding shoots and developing fruitlets (Sawicki *et al.*, 2015) as well as among immature fruit (Sadka *et al.*, 2023) that mediate IFA act to inhibit growth before fruitlets detach from the tree.

Given that auxin plays a fundamental role in mediating cell division and expansion during the growth phase of fruit development (Kumar *et al.*, 2014; Fenn and Giovannoni, 2021), our results suggest that reduced auxin activity in the seed coat and pericarp plays a key role in fruitlet growth arrest (Fig. 4). This potential reduction in auxin activity was also accompanied by an increase in ABA (Fig. 5), which can function as an inhibitor of growth (Chen *et al.*, 2020). Indeed, the seed coat and pericarp of arresting fruitlets exhibited an overall up-regulation of ABA biosynthesis genes during growth arrest. Although ABA concentration did not increase in the embryo during growth arrest, up-regulation of *NCED-like\_2* together with elevated levels of DPA suggest that ABA might have been produced but was rapidly degraded. The significant increase in PA and/or DPA in the maternal organs that we observed suggests that ABA was tightly regulated during growth arrest. It is likely that ABA catabolism

was mediated in part by the up-regulation of *CYP707A1-like* and *CYP707A2-like* at early and/or late stages of growth arrest. These results are consistent with previous studies showing that weakly attached avocado fruitlets in the final stage of abscission display elevated levels of ABA in the pericarp (Adato and Gazit, 1977; Garner and Lovatt, 2016). Further, application of ABA to immature avocado fruit suppresses growth (Moore-Gordon *et al.*, 1998). Taken together, we speculate that an increase in ABA in the maternal organs in response to reduced auxin activity functions to suppress growth. Given that ET and JA signaling can also suppress growth (Dubois *et al.*, 2018; Guo *et al.*, 2018), the increases of ACC and JA/JA-Ile (Figs 6, 7) together with ABA might collectively function to inhibit fruitlet growth when auxin activity is reduced in the seed coat and pericarp.

A potential role of seed-coat senescence in avocado fruitlet abscission

Seed coat senescence is a prominent phenotype displayed in abscised avocado fruitlets (Salazar-García *et al.*, 2013; Fig. 8). To evaluate whether it was activated upstream of fruitlet detachment, we investigated the timing of this degenerative process during fruitlet growth arrest. We found that seed-coat senescence was induced at a late stage of growth arrest and it became visibly apparent when the fruitlets shrank just prior to abscission. This was supported by the fact that molecular markers of senescence such as *SAUR36-like\_2*, *SAG12-like*, *BFN1-like*, and *AAF-like* were induced at a late stage of growth arrest before visible signs of senescence. The overall up-regulation of genes encoding positive and negative regulators of senescence at late growth arrest further indicated that the process was highly regulated. As senescence typically precedes abscission (Patharkar and Walker, 2019; Pautot *et al.*, 2025), our results suggest that seed coat senescence might be the final step that commits the arrested fruitlet to abscission.

While senescence can be induced by stress or physical damage, our results suggested that the degenerative process induced in the seed coat during IFA was triggered by the activation of an age-dependent-like senescence pathway. Competence to undergo fruit senescence or ripening is typically acquired during maturation and is associated with reduced auxin activity together with an increase in ABA (Bower and Cutting, 1988; McAtee *et al.*, 2013; Fenn and Giovannoni, 2021). In addition, ABA derived from maternal organs including the seed coat plays a key role in initiating seed maturation and dormancy (Iwasaki *et al.*, 2022; Kavi Kishor *et al.*, 2022). Further, at a late stage of seed maturation, dormancy is accompanied by a programmed degenerative process in the seed coat, which forms a protective layer around the embryo (Radchuk and Borisjuk, 2014). In our study, the decrease in auxin and increase in ABA in the seed coat and pericarp during fruitlet growth arrest (Figs 4, 5) were strikingly similar to the hormonal changes that occur during fruit maturation. Growth arrest was also accompanied by the activation of a dormancy-like

program of development, as indicated by the up-regulation of ABA signaling genes and down-regulation of *FT-like* in the seed coat and pericarp (Fig. 9). Moreover, the increase in *MFT-like* expression in the embryo and seed coat coincided with the expression of senescence-associated genes in the seed coat at late growth arrest (Supplementary Fig. S13). Together, these changes in hormone and gene expression profiles suggest that the different tissues of the fruitlet, including the seed coat, undergo a maturation-like program of development prior to abscission. Further, in the seed coat this program probably functions to commit this maternal organ to senescence.

Hormones that regulate the onset and progression of senescence and ripening include ABA, ET, and JA (Fenn and Giovannoni, 2021; Ouyang *et al.*, 2025). In our study, we found that ABA, ACC (a proxy for ET), and JA-Ile accumulated in the seed coat at a late stage of growth arrest (Figs 5–7). In addition, their biosynthesis and signaling genes were up-regulated predominantly in the seed coat during growth arrest. Interestingly, fruitlets that cease growth in response to ABA treatment also display a senescence phenotype in the seed coat (Moore-Gordon *et al.*, 1998). In addition, the degeneration of the seed coat and nucellus in fruitlets harvested from excised branches is associated with a burst of ethylene primarily derived from the seed coat (Davenport and Manners, 1982). Thus, these findings further implicate ABA and ET signaling in regulating seed coat senescence. Taken together, we speculate that ABA, ACC, and JA-Ile collectively function to promote the onset and progression of seed coat senescence in response to low auxin activity during late growth arrest and the shrinkage phase, respectively.

Is the mechanism of immature fruit abscission conserved across different tree species?

Studies in apple suggest that IFA is initiated in the cortex by ET, which triggers developmental arrest via seed abortion (Botton *et al.*, 2011; Eccher *et al.*, 2015). In contrast, our results suggested that avocado IFA is initiated by hormone signaling originating in the seed coat, which induces seed-coat senescence and a seed dormancy-like program, collectively suppressing fruitlet growth. Further, we propose that the activation and progression of seed-coat senescence represent the final step in the abscission pathway that triggers the detachment of the fruitlet from the tree. In this context, avocado IFA appears to be mediated by a premature activation of an age-dependent abscission pathway in the seed coat.

The differences between IFA in avocado and apple might be attributable to fundamental differences in fruit structure and development. Avocado is classified botanically as a single-seeded berry in which the fleshy pericarp is derived from the ovary wall (Chanderbali *et al.*, 2013). In contrast, apple is classified as a pome containing multiple seeds, where the edible cortex is primarily derived from the hypanthium of the flower

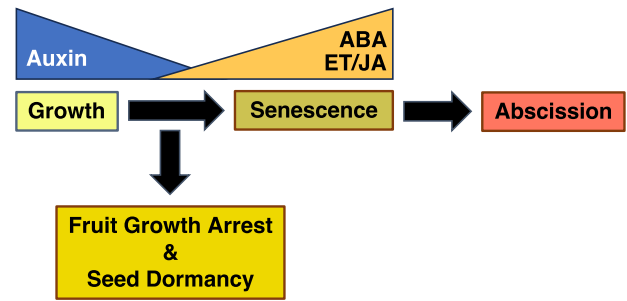


**Fig. 10.** A model for the role of the seed coat in avocado fruitlet abscission. The immature fruit abscission signaling event is initiated in the seed coat by a decrease in auxin activity, which is transmitted to the pericarp and embryo to induce fruitlet growth arrest. As auxin activity declines, increases in ABA, ACC, and JA-Ile collectively act to promote a senescence program of development in the seed coat, which suppresses growth and triggers fruitlet abscission. During the transition to seed-coat senescence, ABA accumulation in the maternal organs induces seed dormancy.

surrounding the ovary (Liu *et al.*, 2020). It is conceivable that the evolutionary and structural differences between a single-seeded berry and multi-seeded pome fruit might have influenced the origin and mechanism of IFA signaling.

In addition to apple and avocado, the hormonal basis of IFA has been studied in other tree crops, including litchi (*Litchi chinensis*) and *Citrus*. Litchi fruit is botanically classified as a single-seeded drupe (Wang *et al.*, 2017), whereas the fruit of *Citrus* is a berry with multiple seeds (Agusti *et al.*, 2026). In litchi, reduced IAA combined with increased ABA and ET in the fruit tissues and/or seed is associated with immature fruit abscission (Kuang *et al.*, 2012; Li *et al.*, 2015; Wang *et al.*, 2024), whereas in *Citrus*, abscission is associated with an increase in ABA and ACC in the fruit tissues (Gómez-Cadenas *et al.*, 2000). While there appears to be some conservation of hormones implicated in IFA across different fruit tree species, the tissue in which signaling is initiated and the developmental mechanism leading to abscission might differ depending on fruit type. Further investigation is required to determine the origin, identity, and integration of IFA hormone signaling in diverse fruit systems.

## Conclusion

Based on our results, we propose a model in which changes in seed-coat hormone signaling determine the fate of developing avocado fruitlets (Fig. 10). In this model, IFA is initiated when the seed coat transitions to a senescence program of development. This developmental transition is itself initiated by a decrease in auxin activity in the seed coat. As auxin levels decline, increased ABA, ACC, and JA-Ile collectively act to suppress growth and activate a senescence program of development in the seed coat at late growth arrest. In addition, our results also suggest that an increase of ABA signaling together with a decrease of *FT-like* expression in the seed coat initiates a dormancy-like program of development in the seed that promotes *MFT* expression in the embryo at a late stage of growth

arrest. Finally, our results indicate that decreased auxin biosynthesis and activity in the seed coat might ultimately limit auxin transport through the pedicel, thus allowing ET and possibly JA-Ile to activate cell separation in the abscission zone. Overall, this working model provides a framework for developing new management practices to reduce summer fruit-drop in avocado.

## Supplementary data

The following supplementary data are available at [JXB](#) online.

**Fig. S1.** Defoliation induces fruitlet growth arrest and abscission.

**Fig. S2.** Experimental design to validate the expression of candidate genes differentially expressed during fruitlet growth arrest.

**Fig. S3.** Validation of gene expression profiling by qRT-PCR for selected differentially expressed genes associated with fruit growth arrest.

**Fig. S4.** Gene expression profiling of auxin biosynthesis and conjugation genes during fruitlet growth arrest.

**Fig. S5.** Differential expression of auxin transport genes during fruitlet growth arrest.

**Fig. S6.** Differential expression of auxin response factors during fruitlet growth arrest.

**Fig. S7.** Gene expression profiling of ABA biosynthesis genes during fruitlet growth arrest.

**Fig. S8.** Gene expression profiling of the *CYCP707A* ABA catabolism genes during fruitlet growth arrest.

**Fig. S9.** Quantification of ABA-GE in normal growing and arresting fruitlets.

**Fig. S10.** Gene expression profiling of ET biosynthesis genes during fruitlet growth arrest.

**Fig. S11.** Gene expression profiling of JA biosynthesis genes during fruitlet growth arrest.

**Fig. S12.** Mean growth rate of fruitlets used to quantify seed-coat senescence.

**Fig. S13.** Gene expression profiling of senescence-associated genes during fruitlet growth arrest.

**Fig. S14.** Differential expression of ET signaling genes during fruitlet growth arrest.

**Fig. S15.** Differential expression of JA signaling genes during fruitlet growth arrest.

**Fig. S16.** Gene expression profiling of ABA signaling genes in response to growth arrest.

**Fig. S17.** Gene expression profiling of dormancy signaling genes in response to growth arrest.

**Fig. S18.** Differential expression of cell-cycle genes during growth arrest.

**Table S1.** Primers used for qRT-PCR in this study.

**Dataset S1.** Annotation of RNA-seq data and GO analysis for differentially expressed genes.

## Acknowledgements

The authors thank Dr Ramesh V. Nair (Director of Bioinformatics, Stanford Center for Genomics and Personalized Medicine) for computational and bioinformatics services. This work utilized computing resources provided by the Stanford Genetics Bioinformatics Service Center. We are very grateful to Phytech for their provision of the dendrometers for the duration of the experiments. We are also grateful to Dr Jean Davidson (Cal Polytechnic State University) for supporting the bioinformatic analyses. This work was made possible by Neil Delroy and Jasper Farms, as well as Ben Dring and Andrew Harty at Costa Group, for providing the team with access to the avocado trees used in our trials. We

thank Drs Vy Nguyen, Sarah Hake, and Ben Trevaskis for critical reading of this manuscript. Lastly, we thank Jacinta Foley and Declan McCauley (Jasper Farms and the Western Australia Department of Primary Industries and Regional Development, respectively) for assistance with the field trials. This work is dedicated to the memory of Reuben Hofshi and Samuel Salazar-Garcia.

## Author contributions

AH and HMS designed the research; AH, MG, PH, and SMM performed the research; HMS, AH, MG, CB, and PH analysed the data; HMS and MG wrote the manuscript.

## Conflict of interest

The authors declare that they have no conflicts of interest in relation to this work.

## Funding

This work was funded by CSIRO and the Hort Innovation project AV16005 titled 'Maximizing yield and reducing seasonal variation', using the avocado research and development levy.

## Data availability

The raw RNA-seq datasets used in this study are available at the CSIRO Data Access Portal (<https://doi.org/10.25919/psft-zg47>).

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