



Tissue-Specific Absciscic Acid, Jasmonic Acid, and Cytokinins Dynamics During On-Tree Ripening of ‘Rojo Brillante’ Persimmon

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Abstract

This study investigated the tissue-specific dynamics of abscisic acid (ABA) and its metabolites, jasmonic acid (JA), and cytokinins (CK) in the apical and basal flesh and the calyx of ‘Rojo Brillante’ persimmon during fruit development and on-tree ripening. Flesh ABA and 7-OH-ABA were high at the beginning of fruit development (60 days after full bloom, DAFB) and showed a transient accumulation at 150–180 DAFB, which was associated with ripening-related changes such as increased external color and firmness loss. The decline in ABA levels at the last ripening stages coincided with the increase in its oxidized metabolites (PA and DPA), indicating active ABA catabolism during late maturation. JA remained low in the flesh until 150 DAFB but rose thereafter in the apical region, suggesting a tissue-specific contribution to texture modulation. CK levels, mainly, *tZ* and *iP* were related to fruit growth. Moreover, the early increase in *tZ* was accompanied by decreases in ABA and 7-OH-ABA, suggesting antagonistic crosstalk between CK and ABA. In the calyx, the peak of ethylene and CO₂ at 120 DAFB coincided with the maximum accumulation of ABA and 7-OH-ABA in this tissue and was linked to the onset of fruit ripening. In contrast to flesh, JA levels in the calyx were markedly high during early development and declined progressively through maturation. CK in the calyx followed distinct patterns throughout fruit maturation. Together, these findings provide a coherent framework for understanding persimmon development and maturation and establish a basis for optimizing harvest timing and postharvest management.

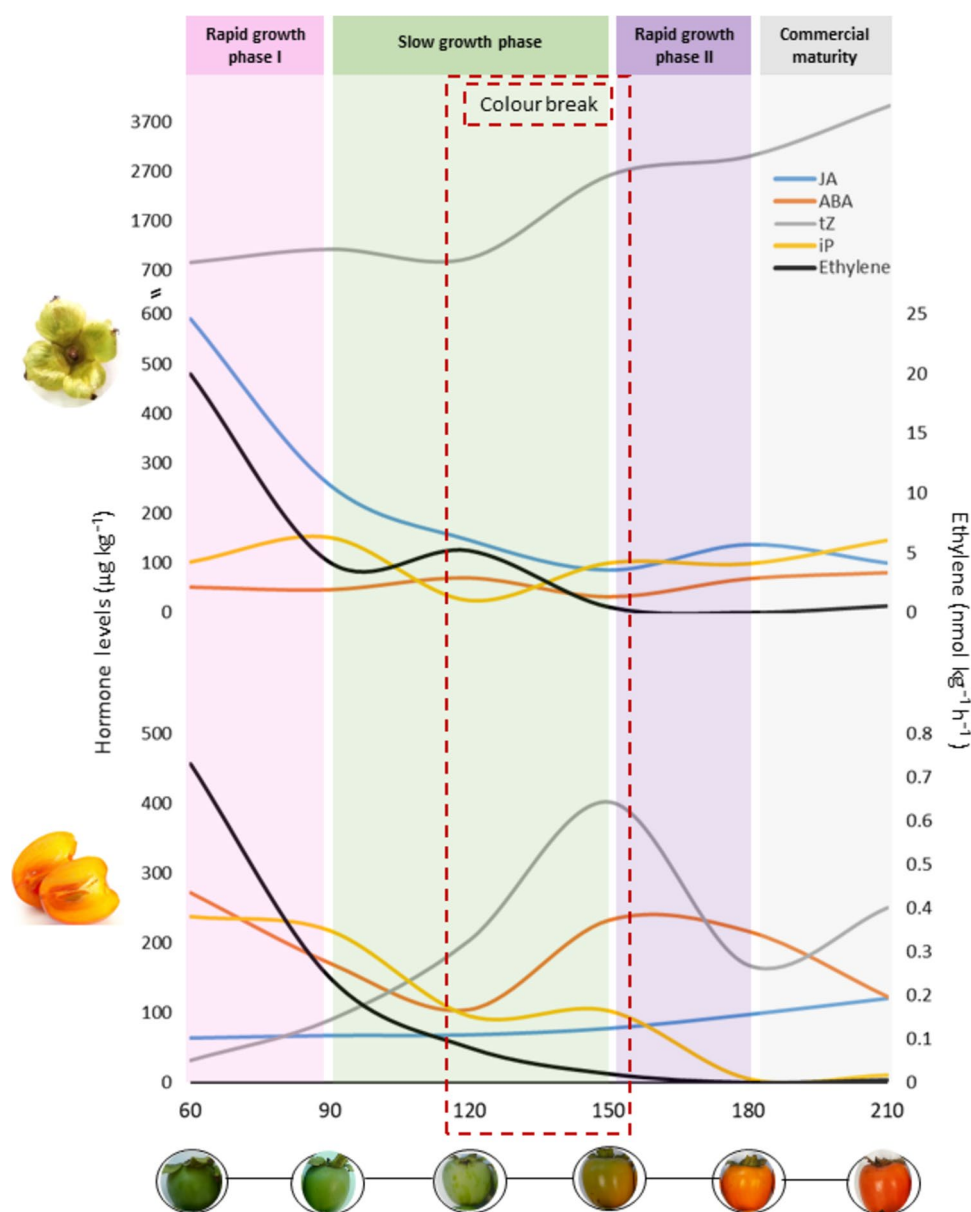
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Graphical Abstract



Keywords *Diospyros kaki thunb.* · Hormonal profile · Fruit development · Calyx

Introduction

Persimmon (*Diospyros kaki* Thunb.), particularly the ‘Rojo Brillante’ cultivar, is a significant fruit crop in Mediterranean regions due to its high commercial value, attractive appearance and favourable sensory characteristics (Salvador et al. 2007). This is an astringent cultivar at maturity stage, which requires to be subjected to a postharvest deastringency treatment before being commercialised with high firmness (Vilhena et al. 2022a). Although persimmon

fruit is classified as climacteric, it exhibits a notably low production of endogenous ethylene during fruit development and ripening (Salvador et al. 2020). Nevertheless, it is highly sensitive to exogenous ethylene, which can rapidly trigger softening and other ripening-related changes (Besada and Salvador 2018). While ethylene is traditionally considered the primary hormonal regulator in climacteric fruit, in non-climacteric species, other phytohormones such as the abscisic acid (ABA) are associated to key mechanisms of fruit development and ripening (Kou et al. 2021).

More recently, an emerging body of work has suggested that different hormones such as ABA, jasmonic acid (JA), and cytokinins (CK), also have significant roles in the development, maturation and ripening of climacteric fruit (Ding et al. 2013; Duan et al. 2015; Huang et al. 2017).

Absciscic acid has been reported to play a key role in the initiation and progression of ripening in climacteric fruit such as apple and tomato (Fernández-Cancelo et al. 2023; Zhang et al. 2009). This hormone not only promotes colour development and flesh softening but also interacts with other hormones, including ethylene, forming an intricate hormonal interplay that fine-tunes the ripening response (Weng et al. 2015). In addition to ABA biosynthesis, its catabolism and conjugation are critical for determining biologically active levels of ABA metabolites within fruit tissues (Nambara et al. 2005). ABA can be conjugated into inactive forms such as ABA-glucose ester (ABA-GE) or degraded via hydroxylation into phaseic acid (PA) and neo-PA. PA, in turn, can be further reduced to dihydrophaseic acid (DPA) (Lai et al. 2020). PA and neo-PA are considered active ABA catabolites because they activate a subset of ABA receptors, although to a much lesser extent than ABA, whereas DPA is biologically inactive (Weng et al. 2015). These pathways dynamically modulate ABA homeostasis, ensuring tissue- and stage-specific hormonal activity. The behaviour of these catabolites and conjugates during persimmon ripening remains poorly explored, particularly with respect to their spatial distribution within the fruit.

Jasmonic acid and CK are also increasingly recognized as key regulators of fruit development and ripening. JA has been associated with defence responses and the modulation of ripening traits (Huang et al. 2017; Wasternack et al. 2014), whereas CK are involved in cell division and fruit development (Kumar et al. 2013). Profiling bioactive CK, such as *trans*-zeatin (*tZ*), *cis*-zeatin (*cZ*), isopentenyladenine (*iP*), and their metabolites (ribosides and nucleotides), can provide insight into their temporal and tissue-specific dynamics during fruit maturation.

Despite significant advances in understanding hormone-mediated processes in other fruit species, information regarding these mechanisms in persimmon remains limited. To elucidate the regulatory roles of plant hormones during persimmon development and ripening, it is essential not only to access their temporal dynamics but also to evaluate their spatial distribution across the fruit. In 'Rojo Brillante', differential accumulation of nutrients has been reported between the apical and basal regions of the flesh, which are closely associated with physicochemical traits such as firmness and colouration (Vilhena et al. 2022a). Furthermore, during ripening, the apical region softens earlier than the basal region, a phenomenon whose underlying physiological basis remains unclear. In addition, the persimmon calyx

has been described as playing a pivotal role in fruit respiration and is considered an important site of gas exchange between the fruit and the external environment throughout development, potentially influencing fruit's metabolic processes (Yonemori et al. 1996). These distinct physiological characteristics among the apical, basal and calyx regions suggest that hormone biosynthesis, metabolism, and signalling may vary spatially within the fruit. A detailed analysis of hormone profiles in these specific tissues could thus offer valuable insights into their distinct contributions to overall fruit growth and ripening. Therefore, this study investigated the tissue-specific dynamics of absciscic acid, jasmonic acid, and cytokinins in 'Rojo Brillante' persimmon during fruit development and on-tree ripening.

Material and Methods

Fruit Material and Experimental Design

Persimmon 'Rojo Brillante' was harvested from the *Instituto Valenciano de Investigaciones Agrarias* (IVIA) experimental orchard, located in Moncada, Valencia, Spain (39°35'18.5"N 0°23'57.3"W). This region has a typically Mediterranean climate, with 400–500 mm of mean annual rainfall and average annual temperatures of 17–18 °C (Riegos IVIA 2026). The soil in this area has a sandy loam to sandy clay loam texture (Vicent et al. 2011).

Harvesting was carried out at monthly intervals across different stages of fruit development. The first harvest took place on 11 July, corresponding to the beginning of growth phase I, approximately 60 days after full bloom (DAFB) (Candir et al. 2009; Badenes et al. 2015). Subsequent harvests were on 1 August (90 DAFB), 1 September (120 DAFB), 5 October (150 DAFB), 3 November (180 DAFB) and 1 December (210 DAFB).

At each harvest point, 30 fruits were randomly harvested from five trees (10 fruits as one biological replicate) and immediately transported to the laboratory for evaluation. Two fruits per replicate were taken to measure CO₂ and ethylene production. The eight remaining fruits were individually measured for weight, size, external colour and flesh firmness. Afterwards, fruit was separated into three parts: calyx, basal flesh half and apical flesh half (Vilhena et al. 2022a). For hormone extraction and determination, three biological replicates per tissue, consisting of tissue from eight fruits were taken. The pooled samples were sliced, frozen in liquid nitrogen and lyophilized in a FreeZone 6 freeze drier system (0.05 bar, -50 °C) (Labconco, Kansas City, MO, USA) in the dark for up to 5 days. Then, they were ground to powder and stored at -20 °C for subsequent analysis.

Physicochemical Evaluation

Weight was determined using a digital balance (model PB3002-S/FACT, Mettler 85 Toledo, Switzerland). Longitudinal and equatorial diameters were measured with a caliper (Mitutoyo 500-267 V-CDL20CP, Japan).

External colour was evaluated using Hunter L, a, b parameters with a colorimeter (Minolta, CR-300, Ramsey, NY, USA) by taking two measurements per fruit on opposite sides of the equatorial zone. Results were expressed as colour index (CI=1000 a/Lb) (Besada and Salvador 2018).

Firmness was measured on the equatorial side of each fruit, after removing the peel, using a texturometer (Instron Corp., mod. 4301, Canton, MA, USA) fitted with an 8-mm diameter probe, following Salvador et al. (2004). Results were expressed in Newton (N) as the force required to break the flesh.

For soluble tannins analysis, flesh from three fruits per replicate was cut and frozen at -20°C until analysis. Determination followed the Folin-Denis method (Taira and Ono 1996) and results were expressed as a percentage of fresh weight.

To determine total soluble solids, juice from three fruits per replicate was obtained using an electric juice extractor and filtered. To avoid the interference of tannins, they were precipitated prior to measurement according to Sugiura et al. (1983), using a 5% polyethyleneglycol solution (w/w) (PEG 6000, Panreac). Measurements were taken with a refractometer (Atagomod. PR1), and results were expressed as a percentage.

Ethylene and CO_2 production were evaluated for whole fruit and for the calyx, which was previously separated from the fruit as described by Fathi-Najafabadi et al. (2021). Fruits and calyces were placed individually into 1 L and 100 mL jars, respectively, sealed and held at 20°C for 24 h. Headspace CO_2 and ethylene concentrations were determined by withdrawing a 1 mL gas sample with a BD Plastipak syringe through a silicone septum and injecting it into a gas chromatograph (model 2000, PerkinElmer, Norwalk, CT, USA) fitted with a Porapak QS 80/100 column. Ethylene and CO_2 production rates were calculated as described by Vilhena et al. (2022b) and expressed as $\text{nmol kg}^{-1} \text{h}^{-1}$ and $\text{mmol kg}^{-1} \text{h}^{-1}$, respectively.

Extraction and Quantification of Plant Hormones

Phytohormones were quantified using the previously prepared freeze-dried, powdered tissue samples. For each of the three biological replicates, 10.0 ± 0.1 mg aliquots were extracted, in triplicate (technical replicates), with 1.0 mL of HPLC-grade methanol: water:formic acid (60:35:5 v/v/v) and 10 μL of internal standards (iSTD) at 100 ng mL^{-1} ,

following García-Pastor et al. (2021) with modifications. The solution was vortex-mixed using at 2,000 rpm for 2 min, placed on ice in the dark for 20 min, and centrifuged at $4,500 \times g$ for 10 min at 4°C . The supernatant was collected and lyophilised overnight. Dried extracts were reconstituted in 1.0 mL of methanol:water:formic acid (1:9:0.1 v/v/v) containing 1 mM ammonium formate, vortex-mixed at 2,000 rpm for 3 min, and centrifuged at $4,500 \times g$ for 10 min at 4°C . The supernatant was filtered through a $0.22\text{-}\mu\text{m}$ PTFE membrane into silanised amber glass vials.

Aliquots (20 μL) were injected into a LC–MS/MS system comprising an Agilent 1200 series HPLC (Agilent, Berkshire, United Kingdom) coupled to a Q-Trap 6500 mass spectrometer (AB Sciex, Framingham, MA, USA), equipped with a Hypersil GOLD C18 column ($50 \times 2.1 \text{ mm}$, $1.9 \mu\text{m}$; Thermo Fisher Scientific, Waltham, MA, USA) maintained at 30°C . Separation was achieved at 0.4 mL min^{-1} using a mobile phase consisting of 0.1% formic acid with 1 mM ammonium formate in water (A) and 0.1% formic acid with 1 mM ammonium formate in methanol (B) with an increasing gradient of B: 5% for 1 min, 40% at 5 min, 95% at 6.5 min (held for 1 min), then returned to 5% at 8.5 min. Quantification used a 10-point calibration curve ($0.5\text{--}500 \text{ ng mL}^{-1}$). Hormones concentrations were expressed as $\mu\text{g kg}^{-1}$ dry weight (DW).

Deuterated and non-deuterated ABA metabolites: (+)-ABA-glucose ester (ABA-GE), (–)-phasic acid (PA), (–)-dihydrophaseic acid (DPA), (–)-neo-PA (neo-PA), and ($\pm$)-7'-OH-hydroxy-ABA (7-OH-ABA) were purchased from the National Research Council of Canada—Plant Biotechnology Institute (Saskatoon, SK, Canada); ($\pm$)-ABA and Jasmonic Acid (JA) were obtained from Sigma-Aldrich (Darmstadt, Germany); and the deuterated and non-deuterated isopentenyladenine (iP), isopentenyladenosine (iPR), isopentenyladenosine-5'-monophosphate (IPRP), trans-zeatin (tZ), cis-zeatin (cZ), trans-zeatin (tZR), cis-zeatin riboside (cZR) and cis-Zeatin riboside-5'-monophosphate (cZRP) were purchased from OlChemIm, Olomouc, Czech Republic.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) to identify significant differences ($p < 0.05$) between sampling times and fruit tissues (apical flesh, basal flesh and calyx), and their interactions. Means were compared using the least significant difference (LSD) test at $p < 0.05$. Analyses were performed with Statgraphics Centurion XVII.I software (Manugistics, Inc., Rockville, MD, USA). Data normality and homoscedasticity were assessed using the Shapiro–Wilk and Levene tests, respectively, prior to ANOVA.

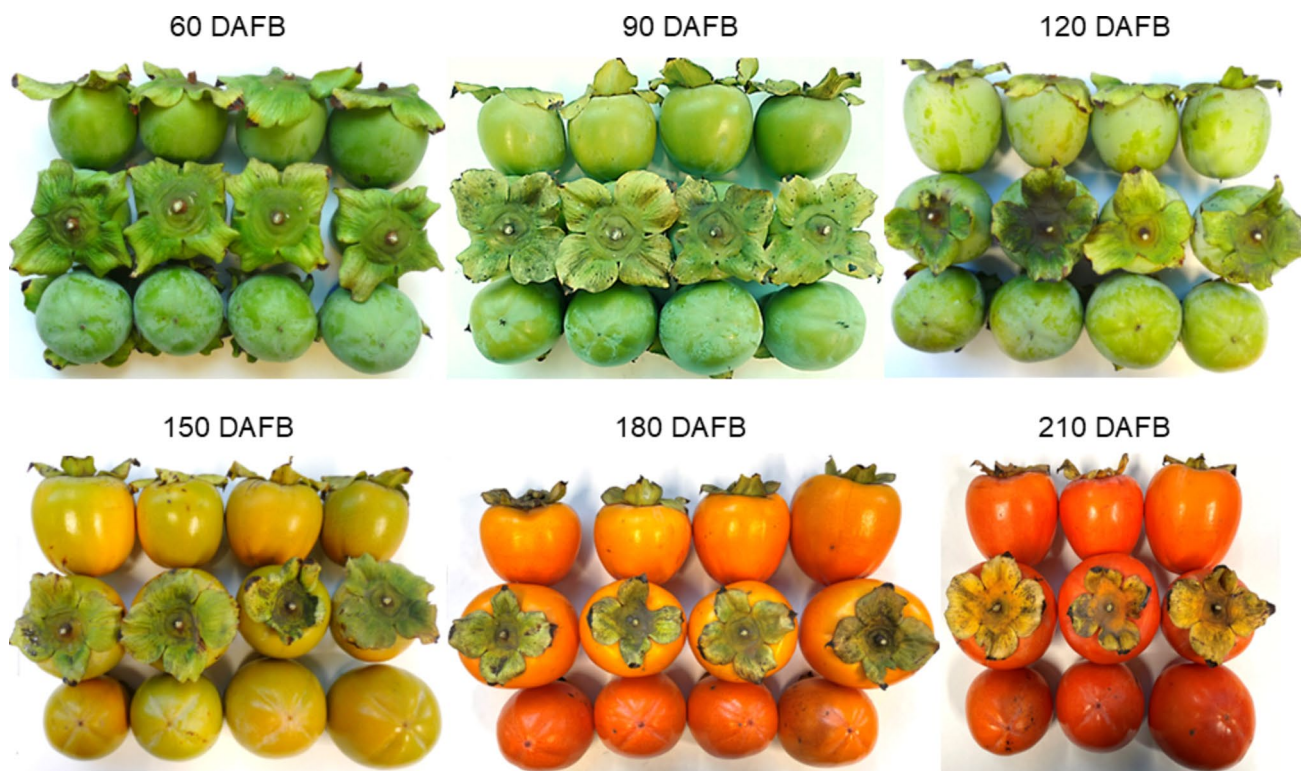


Fig. 1 Captured images of 'Rojo Brillante' persimmon throughout fruit development (DAFB=Days after full bloom)

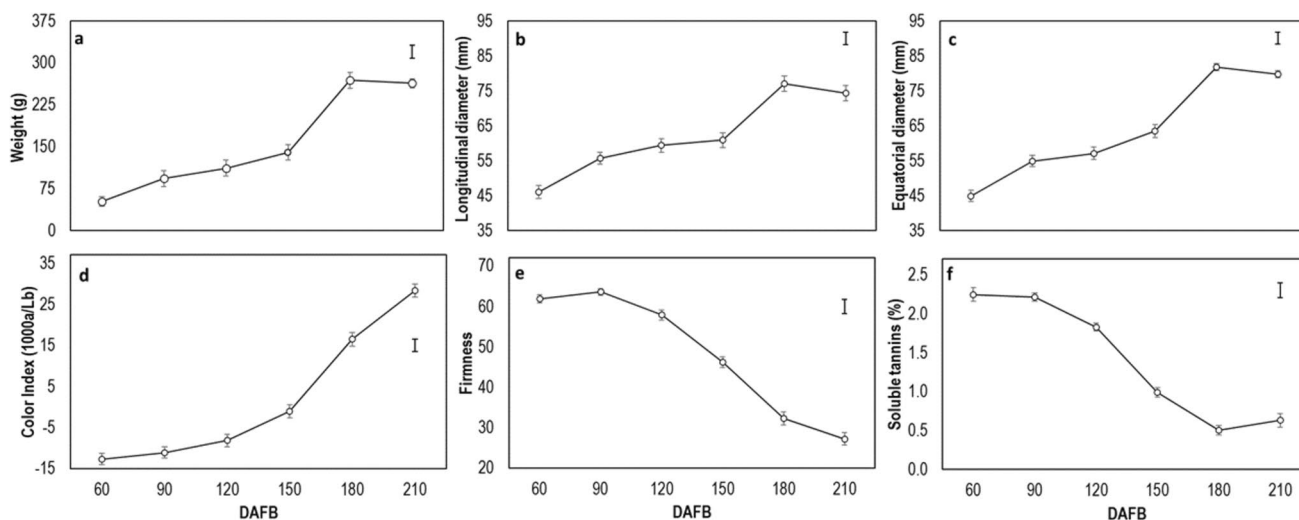


Fig. 2 Physicochemical characterization of persimmon fruit over time. Weight (a), longitudinal diameter (b), equatorial diameter (c), colour (d), firmness (e), soluble tannins (f) of 'Rojo Brillante' persimmon throughout fruit development (DAFB=Days after full bloom). Ver-

tical bars at each data point indicate the standard error of the mean (SE). The standalone vertical bar on the right-hand graphs corner represents the least significant differences (LSD) interval ($p \leq 0.05$) for each maturity stage

Results

Fruit Physicochemical Parameters

During the development of 'Rojo Brillante' persimmon, significant changes in physicochemical attributes were

observed, as reflected in fruit appearance (Fig. 1). At the initial stage (60 DAFB), fruit held an average weight of 52 g and longitudinal and equatorial diameters of approximately 46 mm (Fig. 2a-c). These parameters increased progressively, reaching 265 g in weight and 74 mm and 80 mm in longitudinal and equatorial diameters, respectively, by the last stage (210 DAFB).

External colour also changed markedly during maturation. At 60 DAFB, fruit exhibited a colour index (CI) of -13, corresponding to a green hue (Fig. 2d). As ripening advanced, a continuous increase in CI values occurred, culminating in an orange-red tone (CI=28.3) by 210 DAFB. This colour development was accompanied by a pronounced decline in flesh firmness over the evaluation period. At 60 DAFB fruit had a high firmness, of 62 N, but decreased to 27.2 N by 210 DAFB (Fig. 2e). The sharpest reduction occurred between 120 and 180 DAFB, coincident with the transition in skin colouration. Simultaneously, soluble tannin (ST) content fell from an initial value of 2.2% to 0.6% by 180–210 DAFB (Fig. 2f).

In terms of fruit gas exchange, when measurements were performed on whole fruit with the calyx attached, ethylene production was highest at 60 DAFB ($0.73 \text{ nmol kg}^{-1} \text{ h}^{-1}$) and decreased thereafter, reaching minimal levels ($<0.05 \text{ nmol kg}^{-1} \text{ h}^{-1}$) from 150 DAFB onwards (Fig. 3a). CO_2 production also showed an early maximum at 60 DAFB ($53 \text{ mmol kg}^{-1} \text{ h}^{-1}$), followed by an approximately 2.2-fold decline by 90 DAFB (Fig. 3b). Transient CO_2 peaks ($\sim 30 \text{ mmol kg}^{-1} \text{ h}^{-1}$) were detected at 120 and 180 DAFB, before reach a minimal production, of $2 \text{ mmol kg}^{-1} \text{ h}^{-1}$, at 210 DAFB.

Importantly, measurements performed on detached calyces revealed a substantially greater capacity for ethylene and CO_2 than the whole fruit. Calyx ethylene production reached $20 \text{ nmol kg}^{-1} \text{ h}^{-1}$ at 60 DAFB, approximately 20-fold higher than in whole fruit (Fig. 3c). This production rapidly decreased to $4.2 \text{ nmol kg}^{-1} \text{ h}^{-1}$ by 90 DAFB, slightly increased at 120 DAFB, to drop again to values below $0.1 \text{ nmol kg}^{-1} \text{ h}^{-1}$ from 150 DAFB onward. Similarly, calyx CO_2 production was high at 60 DAFB ($260 \text{ mmol kg}^{-1} \text{ h}^{-1}$), peaked at $540 \text{ mmol kg}^{-1} \text{ h}^{-1}$ at 120 DAFB, and then declined steadily to near-zero values by 210 DAFB (Fig. 3d).

Persimmon Phytohormones

Abscissic Acid

Changes in abscisic acid (ABA) metabolism during persimmon development revealed coordinated yet tissue-specific dynamics. In the flesh, basal ABA at 60 DAFB was approximately twice that of the apical region (366 and $155.6 \mu\text{g kg}^{-1}$, respectively), followed by a decline in both tissues to similar levels at 120 DAFB (Fig. 4a). A transient accumulation was observed at 150–180 DAFB, after which ABA decreased by 210 DAFB. The metabolic product of ABA oxidation, 7-OH-ABA, mirrored this pattern, decreasing

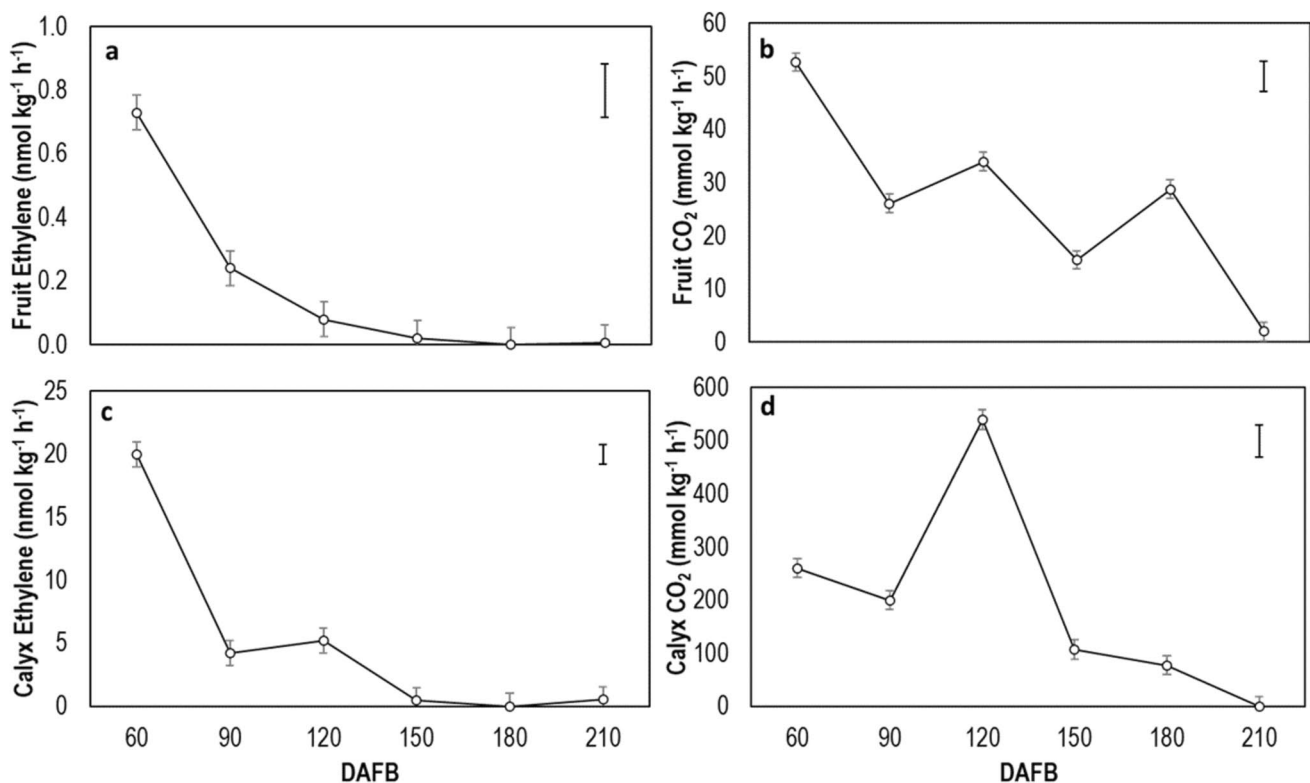


Fig. 3 Ethylene and CO_2 production by whole fruit (a, b) and by detached calyx (c, d) of 'Rojo Brillante' persimmon throughout fruit development (DAFB=Days after full bloom). Vertical bars at each

data point indicate the standard error of the mean (SE). The standalone vertical bar on the right-hand graphs corner represents the least significant differences (LSD) interval ($p \leq 0.05$) for each maturity stage

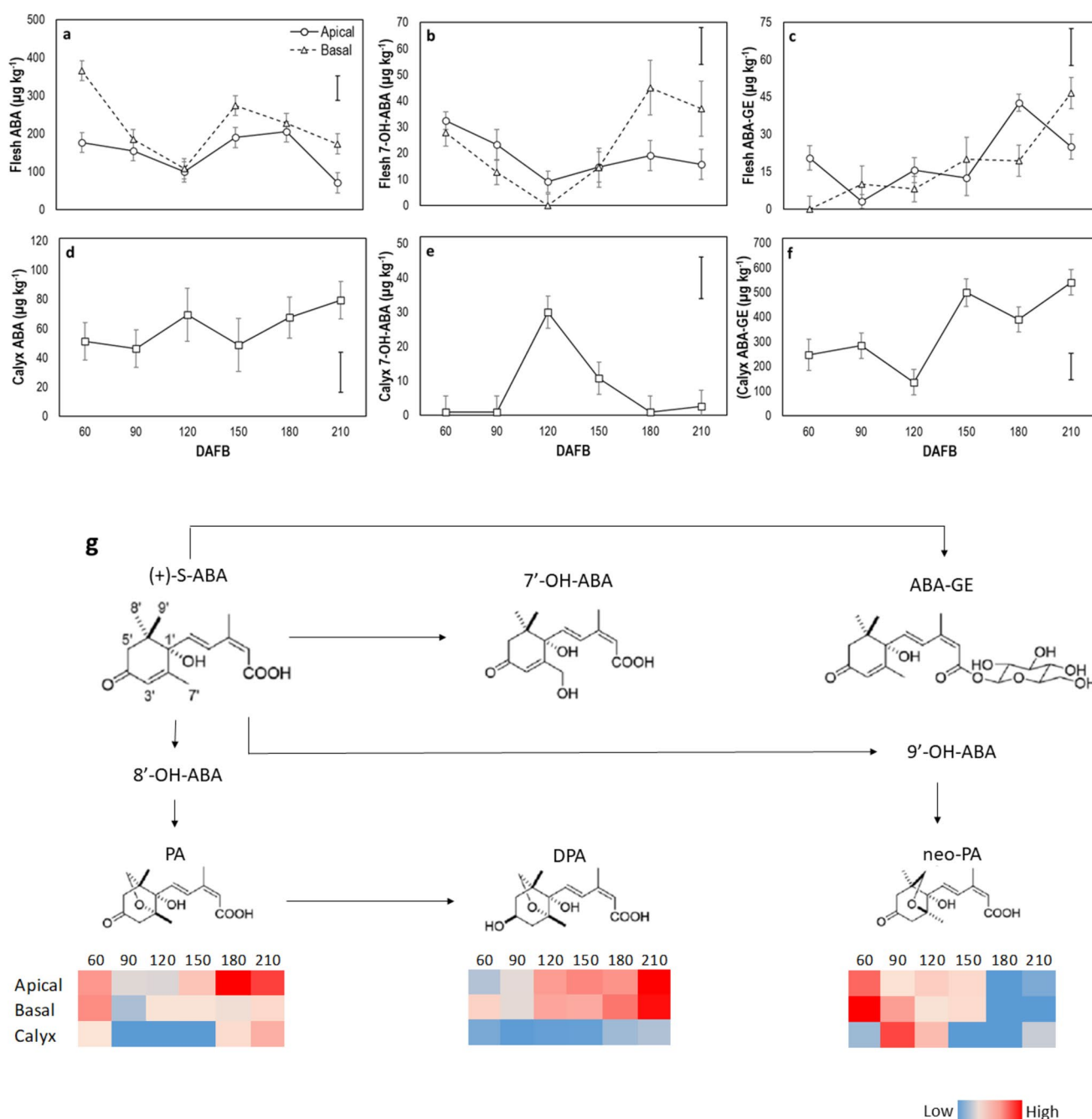


Fig. 4 Absciscic acid metabolism. Absciscic acid (ABA) and its metabolites ($\mu\text{g kg}^{-1}$) were quantified in apical flesh, basal flesh (a-c,g) and calyx tissue (d-g) of 'Rojo Brillante' persimmon throughout fruit development (DAFB=Days after full bloom). Vertical bars at each data point indicate the standard error of the mean (SE) (a-f); whilst

the standalone vertical bar on the right-hand side graphs corner represents the least significant differences (LSD) intervals ($p \leq 0.05$) for the interaction tissue type $\times$ maturity stage (a-c) and for each maturity stage (d-f)

from 60 to 120 DAFB and rising thereafter, although differences between flesh regions were only evident at later stages (180–210 DAFB), with the basal flesh showing the highest concentrations (Fig. 4b). The conjugated ABA-glucose ester (ABA-GE) remained low in flesh during fruit development, with no major differences between parts, and increased progressively during fruit maturation (Fig. 4c).

The calyx, on the other hand, displayed a distinct profile. Calyx ABA concentrations were relatively stable across stages, with modest increases around 120 and again at 210 DAFB ($\sim 75 \mu\text{g kg}^{-1}$; Fig. 4d). Calyx 7-OH-ABA was very low at the first two stages, peaked at $30 \mu\text{g kg}^{-1}$ at 120 DAFB, and then declined to near-zero by the final stage (Fig. 4e).

ABA-GE in the calyx decreased up to 120 DAFB, followed by a sharp rise to $500 \mu\text{g kg}^{-1}$ by 210 DAFB (Fig. 4f).

Oxidised ABA metabolites, phaseic acid (PA) and neophaseic acid (neo-PA), and the reduced metabolite dihydrophaseic acid (DPA) were also quantified (Fig. 4g). In the flesh, PA declined from $76 \mu\text{g kg}^{-1}$ at 60 DAFB to $13 \mu\text{g kg}^{-1}$ at 90 DAFB. While basal PA then remained low through 210 DAFB, apical PA increased gradually, peaking at $156 \mu\text{g kg}^{-1}$ at 180 DAFB. DPA was the most abundant ABA catabolite in the flesh, accumulating steadily from $500 \mu\text{g kg}^{-1}$ at 60 DAFB to $2,065 \mu\text{g kg}^{-1}$ at 210 DAFB, with no substantial differences between flesh parts. Neo-PA declined progressively in both apical and basal flesh, from $6.0 \mu\text{g kg}^{-1}$ at 60 DAFB to near-zero by 210 DAFB.

In the calyx, PA remained low throughout the study period, with only a slight increase from 180 DAFB onwards, reaching $57 \mu\text{g kg}^{-1}$ at 210 DAFB. Calyx DPA levels were markedly lower than in the flesh. Similar to PA, an increase in DPA was only observed from 180 DAFB, reaching $460 \mu\text{g kg}^{-1}$ at the final stage. Neo-PA in the calyx peaked at 90 DAFB ($12 \mu\text{g kg}^{-1}$) and then declined to undetectable levels at the late developmental stages.

Jasmonic Acid

Endogenous JA remained very low in both apical and basal flesh during the early stages of development (60–150 DAFB), at around $60 \mu\text{g kg}^{-1}$ (Fig. 5a). From 180 DAFB onwards, JA increased in the apical region, reaching $175 \mu\text{g kg}^{-1}$ at 210 DAFB. By contrast, the calyx showed markedly higher JA at 60 DAFB, approximately tenfold greater than in the flesh, followed by a sharp decline that stabilised from 120 DAFB onwards (Fig. 5b).

Cytokinin

Eight cytokinin (CK) metabolites were identified and quantified across the apical flesh, basal flesh and calyx during persimmon development and ripening. These comprised CK bases (*tZ*, *cZ* and *iP*), ribosides (*tZR*, *cZR* and *iPR*) and nucleotides (*cZRP* and *iPRP*). The most abundant forms in all three tissues were the free bases *tZ*, *cZ* and *iP*.

In both flesh areas, *tZ* increased progressively from 60 DAFB, peaked at $200 \mu\text{g kg}^{-1}$ by 120 DAFB, declined at 180 DAFB, and rose again to $280 \mu\text{g kg}^{-1}$ at 210 DAFB (Fig. 6a). In contrast, *cZ* remained at trace levels ($\sim 5 \mu\text{g kg}^{-1}$) until 180 DAFB, when the basal flesh increased to $13 \mu\text{g kg}^{-1}$, while the apical flesh remained low (Fig. 6b). *iP* showed the greatest difference between flesh areas: basal flesh started at $400 \mu\text{g kg}^{-1}$ at 60 DAFB and declined to match the apical flesh ($50 \mu\text{g kg}^{-1}$) by 120 DAFB, whereas apical *iP* stayed relatively constant throughout fruit development (Fig. 6c). Riboside and nucleotide forms followed subtler trajectories (Fig. 6d–h). *Trans*-zeatin riboside (*tZR*) rose only in basal flesh during the earliest and latest stages, with no significant change in apical flesh. *Cis*-zeatin riboside (*cZR*) declined gradually in both regions, from $1.9 \mu\text{g kg}^{-1}$ at 60 DAFB to $1.5 \mu\text{g kg}^{-1}$ at 210 DAFB. Isopentenyladenosine (*iPR*) exhibited high values in apical flesh at 60 and 210 DAFB, close to $6.0 \mu\text{g kg}^{-1}$, but remained stable, with $3.5 \mu\text{g kg}^{-1}$ in basal tissue. Finally, *cZRP* showed no variation across flesh regions or stages, remaining around $65 \mu\text{g kg}^{-1}$, whereas *iPRP* decreased progressively with maturation, with apical levels being slightly higher than basal, mainly at 60 and 210 DAFB.

In the calyx, CK concentrations far exceeded those in the flesh, with *tZ* approximately 100-fold higher. From 120 DAFB onwards, *tZ* accumulated steadily, reaching $4,023 \mu\text{g kg}^{-1}$ by 210 DAFB (Fig. 7a). Calyx *cZ* was also

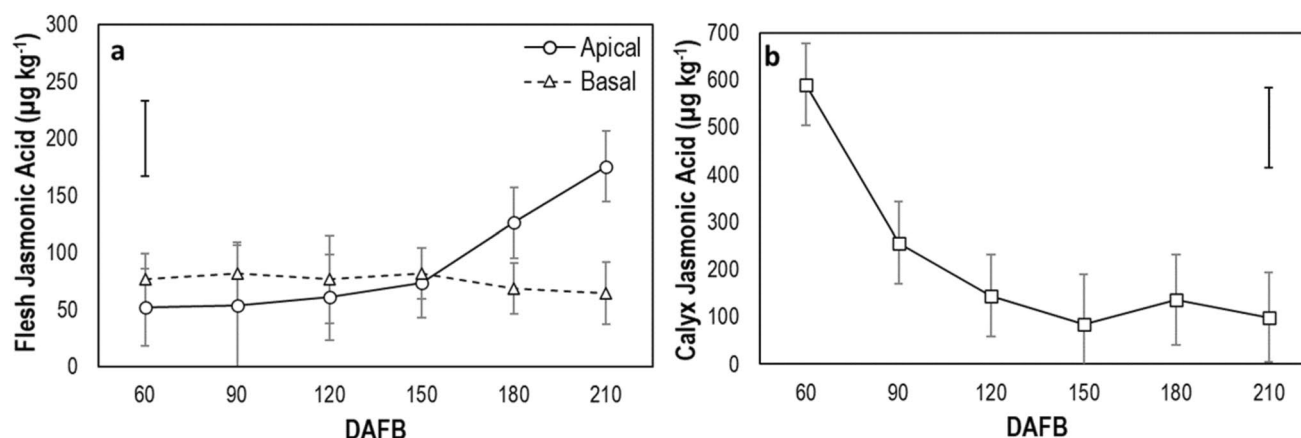


Fig. 5 Jasmonic acid metabolism. Jasmonic acid ($\mu\text{g kg}^{-1}$) quantified in apical flesh, basal flesh (a) and calyx tissue (b) of ‘Rojo Brillante’ persimmon throughout fruit development (DAFB=Days after full bloom). Vertical bars at each data point indicate the standard error of

the mean (SE). The standalone vertical bar on the graphs corner represents the least significant differences (LSD) intervals ($p \leq 0.05$) for the interaction tissue type $\times$ maturity stage (a) and for each maturity stage (b)

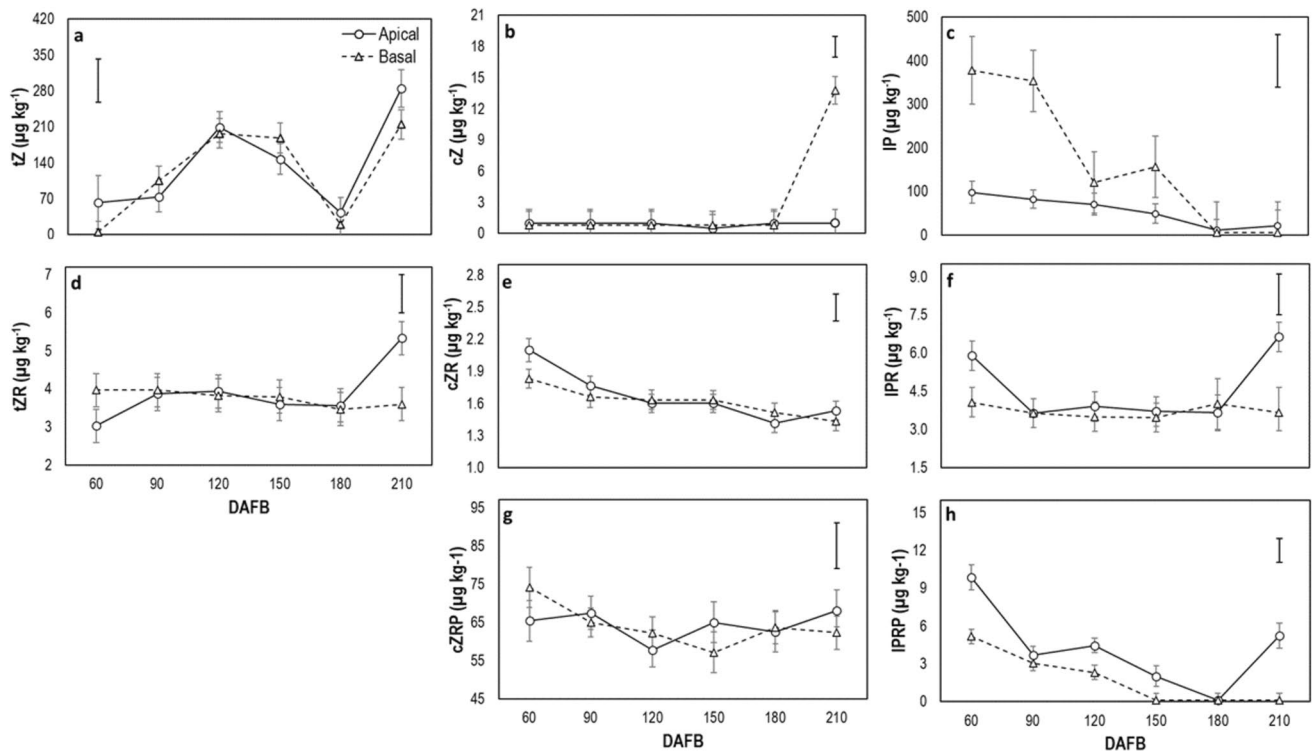


Fig. 6 Cytokinins metabolism in fruit flesh. Cytokinins and its metabolites ($\mu\text{g kg}^{-1}$) were quantified in apical flesh and basal flesh of ‘Rojo Brillante’ persimmon throughout fruit development (DAFB=Days after full bloom). Vertical bars at each data point indicate the standard

error of the mean (SE). The standalone vertical bar on the graphs corner represents the least significant differences (LSD) interval ($p \leq 0.05$) for the interaction tissue type $\times$ maturity stage

high during early development ($\sim 50 \mu\text{g kg}^{-1}$), declined to a minimum at 120 DAFB, and then increased again to nearly $100 \mu\text{g kg}^{-1}$ at 180–210 DAFB (Fig. 7b). iP decreased from $100 \mu\text{g kg}^{-1}$ at 60 DAFB to $25 \mu\text{g kg}^{-1}$ at 120 DAFB, before rising at later stages (Fig. 7c). As in the flesh, riboside and nucleotide forms in the calyx exhibited moderate fluctuations (Fig. 7d–h). Both tZR and cZR increased slightly after 120 DAFB, reaching 5.3 and $2.6 \mu\text{g kg}^{-1}$, respectively, at 210 DAFB. iPR peaked at $5.5 \mu\text{g kg}^{-1}$ at 120 DAFB, then declined to values similar to early development (around $4 \mu\text{g kg}^{-1}$). cZRP showed little change across maturation, remaining around $70 \mu\text{g kg}^{-1}$, while iPRP decreased overall, with pronounced drops at 90 and 150 DAFB interrupted by a transient rise at 120 DAFB.

Discussion

Physicochemical Changes and Ripening Dynamics During Persimmon Fruit Development

Climacteric fruit are characterised by co-occurring respiration rate and ethylene peaks (Wang et al. 2022). However, although classified as climacteric, persimmon is frequently described as an atypical climacteric fruit since endogenous

ethylene production throughout development and ripening is low compared with typical climacteric species, while sensitivity to exogenous ethylene remains high (Nakano et al. 2003; Salvador et al. 2020). In the present study, the progressive decrease in ethylene production from 60 DAFB to undetectable levels from 150 DAFB onwards suggests that, in persimmon, ethylene plays a more prominent role in developmental processes than in on-tree maturation and ripening. For ‘Rojo Brillante’, a small, transient rise in ethylene has been reported at a stage corresponding approximately to 150–180 DAFB (Salvador et al. 2007), a window that coincides in our study with substantial increases in fruit weight and diameter that define persimmon rapid growth phase II (Candir et al. 2009; Yonemori et al. 1996). In the present work, however, this transient ethylene rise was not captured, likely owing to the sampling interval. On the other hand, the high ethylene values detected at the earliest stages correspond to persimmon rapid growth phase I. Previous studies further indicate that ethylene can regulate other aspects of fruit development, such as fruit set and growth, in addition to ripening-related processes (Huang et al. 2022, 2024).

With respect to respiration, the slight increase detected at 120 DAFB likely reflects the onset of physiological changes, including the marked decline in flesh firmness and ST concentration, as well as significant changes in external

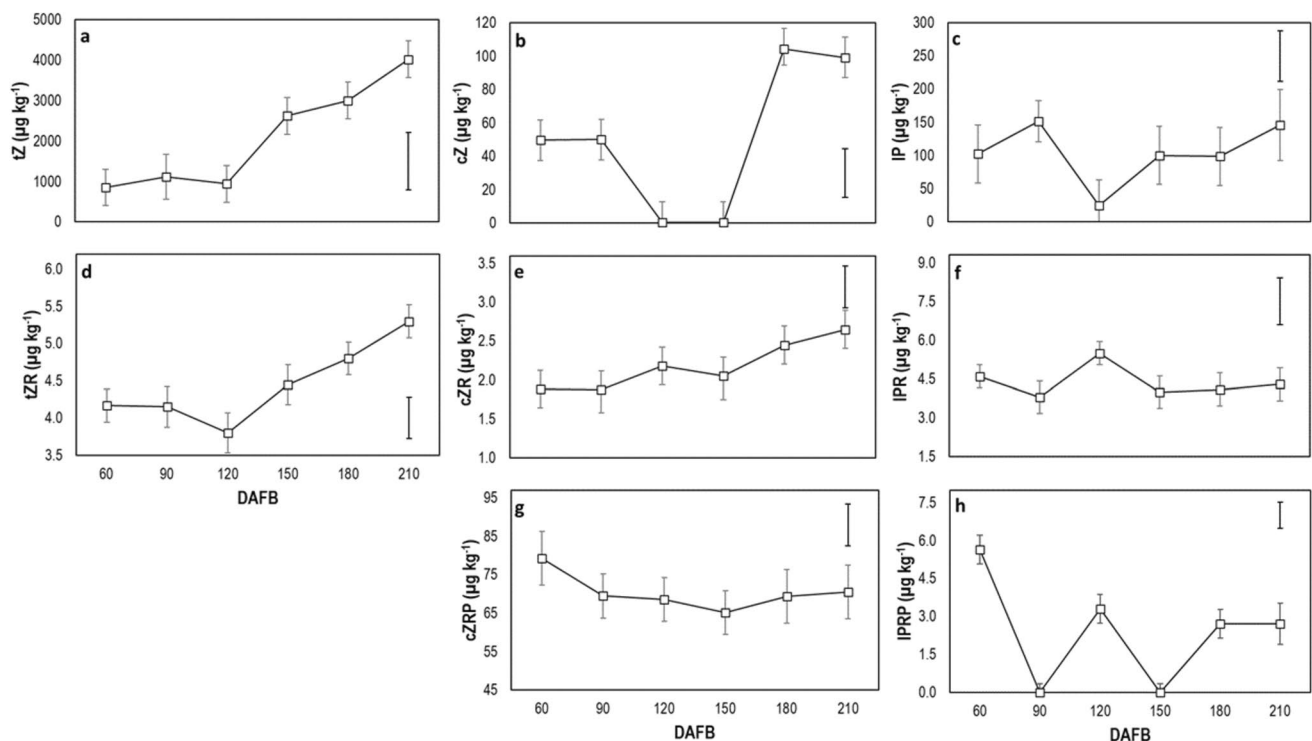


Fig. 7 Cytokinins metabolism in fruit calyx. Cytokinins and its metabolites ($\mu\text{g kg}^{-1}$) quantified in calyx tissue of 'Rojo Brillante' persimmon throughout fruit development (DAFB=Days after full bloom). Vertical bars at each data point indicate the standard error of the mean

(SE). The standalone vertical bar shown on the graphs corner represents the least significant differences (LSD) interval ($p \leq 0.05$) for each maturity stage

colouration from this stage onwards. These metabolic transitions may transiently elevate respiration due to increased cellular activity associated with the initiation of ripening-related processes (Saltveit 2019). Moreover, CO_2 production in persimmon has been reported to increase during the final stages of development (Nakano et al. 1998), which aligns with the CO_2 peak observed at 180 DAFB.

Persimmon fruit possesses a prominent, green, four-lobed calyx that encloses the fruit basal region. In the present study, calyx weight ranged from 3.08 g (60 DAFB) to 4.05 g (210 DAFB), representing 7.02% and 1.06% of total fruit weight, respectively (data not shown). This is in agreement with previous reports indicating that at maturity stage the calyx represent ~1.4% of total fruit weight in persimmon (Murali 2023). Persimmon calyx has been proposed as the main pathway for gas diffusion between the pericarp and the atmosphere, playing an important role in fruit physiological regulation (Besada et al. 2016; Nakano et al. 2003; Yonemori et al. 1996). This is attributed to the high stomatal density on the calyx lobes, while the fruit surface lacks them (Kitagawa and Glucina 1984; Woolf & Ben-Arie 2011). During advanced ripening, calyx senescence progresses from the apical towards the basal portion of the lobes and is associated with desiccation and browning, resembling leaf senescence, which involves chloroplast degradation

and chlorophyll catabolism (Lim et al. 2007). In 'Rojo Brillante', calyx senescence has been linked to overall fruit ripening: a decrease in chlorophyll fluorescence parameters in persimmon calyx have been shown to correlate with fruit external colour evolution and firmness loss during ripening and were subsequently proposed as a non-invasive tool for estimating harvest time and fruit quality (Fathi-Najafabadi et al. 2021).

In the present study, calyx ethylene production was approximately 20-fold higher than in whole fruit, and CO_2 production was nearly fivefold higher. Ethylene declined sharply from 60 to 90 DAFB, followed by transient increase at 120 DAFB that coincided with a pronounced increase in CO_2 production. The ethylene and respiratory activity in the calyx at this stage appears to be associated with enhanced physicochemical changes at the onset of colour break, including accelerated firmness loss and a marked decrease in ST content. Notably, stress-induced increases in calyx ethylene, such as those triggered by chloride treatments, have been reported to accelerate fruit ripening, leading to enhanced colouration and firmness loss (Besada et al. 2016). Although the higher ethylene production in the calyx compared with the fruit could be considered, in part, a consequence of tissue excision, the magnitude of the difference and its clear developmental pattern suggest that ethylene

levels in the calyx primarily reflect a physiological feature of this organ.

In persimmon, it was previously reported that ethylene biosynthesis can be initiated in the calyx which could then diffuse to other fruit tissues, acting as a secondary signal which in turn suggests a calyx–fruit communication via signalling (Nakano et al. 2003). Recent studies in pear further highlight this concept, as calyx excision alters fruit development and is associated with marked changes in hormone signalling pathways, consistent with hormone redistribution and calyx-driven regulation of fruit growth (Jiao et al. 2025; Yu et al. 2025).

Abscisic Acid is Linked to Persimmon Development and on-Tree Ripening

Absciscic acid is a key phytohormone that plays a central role in plant development and physiological regulation. It is widely distributed across plant tissues and organs, where it regulates essential processes such as seed dormancy (Ali and Moon 2025), leaf senescence (An et al. 2021), stomatal opening/closure (Hsu et al. 2021), and responses to abiotic stress (Lim et al. 2022). This hormone has been identified as a crucial regulator of ripening, with a particularly prominent role in non-climacteric fruit, such as grape and cherry, in which increased ABA is associated with the enhancement of key ripening-associated changes, including colour development, softening and sugar accumulation (Palma et al. 2011; Qi et al. 2021). More recently, studies have highlighted ABA's contribution to ripening in climacteric fruit, mainly through crosstalk with ethylene and other hormonal pathways, although the extent and mechanisms remain to be fully understood (Fernández-Cancelo et al. 2023; Kou et al. 2021).

The high levels of ABA and its active oxidative derivative 7-OH-ABA at the earliest stage (60 DAFB) align with findings in ‘Hanagosho’ persimmon and have been associated with the promotion of fruit growth and development (Duan et al. 2015). In other climacteric fruit, such as tomato, evidence indicates that ABA is required for early fruit growth, promoting basal ethylene biosynthesis in System 1 (Kou et al. 2021; Weng et al. 2015).

The elevated ABA and 7-OH-ABA at early stages may also be linked to the high ST content observed at that time. In persimmon, ABA has been implicated in regulating phenolic metabolism via modulation of *DkMYB4*, a transcription factor that controls tannin biosynthesis (Nishiyama et al. 2013; Gupta et al. 2022). In non-climacteric fruit such as grape, pre-harvest ABA application has been reported to enhance fruit phenolic accumulation (Cantín et al. 2007; Sandhu et al. 2011).

On the other hand, endogenous ABA and 7-OH-ABA accumulation at 120–150 DAFB coincided with significant ripening-related changes, including colour development. A peak in ABA at the onset of ripening has likewise been reported in ‘Hanagosho’ persimmon (Duan et al. 2015). This ABA accumulation has been linked to carotenoid metabolism, the main pigment responsible for the characteristic orange-red colouration of persimmon (Jia et al. 2018). This regulation occurs through *9-cis-epoxycarotenoid dioxygenases* (*NCEDs*): *NCED* upregulation elevates ABA, whereas downregulation favours carotenoid accumulation. In parallel, ABA 8'-hydroxylases (*CYP707As*) catalyse ABA catabolism. Thus, the balance between *NCED*-driven biosynthesis and *CYP707A*-mediated degradation governs ABA levels and, indirectly, carotenoids during ripening (Wang et al. 2022).

In ‘Rojo Brillante’, external skin colour is strongly and negatively correlated with flesh firmness, making colour a useful non-destructive indicator of fruit texture (Salvador et al. 2006). Consistent with this, the firmness loss from 150 DAFB may be regulated by rising ABA, which can induce transcription factors involved in cell-wall degradation. In sweet cherry, ABA-induced *PavNAC56* directly activates cell-wall-remodeling gene families, such as polygalacturonases (*PavPG2*), expansins (*PavEXPA4*), pectate lyases (*PavPLI8*) and endo- β -1,4-glucanases/cellulases (*PavCEL8*) (Qi et al. 2021). Likewise, the ABA-responsive factor *PavDof6* upregulates cell-wall-degrading genes such as *PavPME44*, *PavPLI8*, *PavXTH31* and *PavXTH26*, forming a feedback mechanism for ABA-mediated softening.

In the late developmental stages (180–210 DAFB), despite continued colour development and firmness decline, ABA concentrations decreased, particularly in the apical region, while PA progressively accumulated in the same tissue. This pattern indicates active oxidative catabolism of ABA during the ripening phase. Likewise, the increasing levels of DPA, the most abundant ABA catabolite detected, in both apical and basal regions further support sustained ABA degradation in the final stages of maturation and ripening. Similar trends have been observed during the ripening of both climacteric and non-climacteric fruit, such as flat peach and strawberry (García-Pastor et al. 2021; Figueiroa et al. 2021).

Jasmonic Acid, Cytokinins and Hormonal Crosstalk During Persimmon Maturation

Jasmonic acid has been shown to contribute to ripening regulation in both climacteric and non-climacteric crops, including cherry (Fresno and Munne-Bosch 2021), citrus (Zhang et al. 2014) and cape gooseberry (Álvarez-Flórez et al. 2017), by promoting colour development and flesh

softening. This hormone also plays an important role in up-regulating the expression of senescence-associated genes (Huang et al. 2017). In the present study, although JA levels remained low until 150 DAFB, a marked increase was observed in the apical region at 180–210 DAFB, coincident with a reduction in ABA. A potential antagonistic interaction between ABA and JA during fruit development has been proposed. In apple, exogenous Methyl jasmonate (MeJA) applied at the climacteric stage significantly reduced endogenous ABA, suggesting that jasmonates may suppress ABA biosynthesis during ripening, independently of ethylene signalling (Kondo et al. 2001). Similarly, in pear, Wu et al. (2025) reported antagonism between ABA and MeJA in the regulation of anthocyanin metabolism, indicating that JA signalling can counteract ABA pathways to promote pigmentation and ripening. Supporting this pattern, Garrido-Bigotes et al. (2018) proposed that JA negatively regulates ABA signalling during development and ripening of non-climacteric strawberry. The rise in JA in the apical flesh from 150 DAFB coincides with the onset of persimmon ripening, when senescence processes and softening become prominent. Interestingly, in ‘Rojo Brillante’ softening appears to initiate in the apical region, suggesting that JA may play a localised, tissue-specific role in modulating structural changes in this flesh part.

Cytokinins, on the other hand, act as key developmental signals during early fruit development, promoting cell division and expansion, regulating fruit set and influencing final fruit size (Kumar et al. 2013). Among CK bases, iP and *tZ* are generally considered the major bioactive forms, while *cZ* is typically less prominent (Su et al. 2021). This pattern was confirmed in the present study, where iP and *tZ* concentrations in the flesh were consistently higher than *cZ*. High iP in the basal flesh at 60 DAFB coincided with the first rapid growth phase, supporting its active role in cell division and early tissue expansion. In contrast, *tZ* progressive increase during early maturation (60–120 DAFB), indicate a shift in CK profile as development progressed. A similar trend has been reported in tomato, where iP peaks around anthesis and declines thereafter, likely due to conversion or degradation, while *tZ* increases via up-regulation of CK biosynthetic genes, such as *SIPT1*, *SIPT2*, *SICYP735A1*, *SICYP735A2* and *SILOG2* (Matsuo et al. 2012).

Cytokinins also regulate cell-wall-modifying enzymes and contribute to cell enlargement in kiwifruit, where they supersede auxin as the primary phytohormone affecting fruit growth (Nardoza et al. 2020). Evidence from bottle gourd (calabash) likewise suggests coordination between CK, auxin and gibberellins during fruit development, with differentially expressed genes related to CK and auxin biosynthesis and metabolism identified across cultivars of different sizes (Zhang et al. 2020).

Antagonistic interactions between CK and ABA during maturation have also been proposed. According to McAtee et al. (2013), auxin, CK, gibberellins and brassinosteroids dominate during fruit set and early development, while ABA levels are comparatively low. Conversely, the onset of maturation is characterised by reduced auxin transport and enhanced ABA and ethylene biosynthesis. In agreement with this model, in the present study the increase in *tZ* during early development was accompanied by a decline in both ABA and 7-OH-ABA. From 150 DAFB onwards, *tZ* decreased as ABA and its oxidised derivative (7-OH-ABA) began to accumulate, coinciding with the initiation of ripening processes.

In the present study, a direct correlation between ethylene, ABA and JA was not evident in the flesh, likely due to the very low ethylene production in this tissue. Nevertheless, low ethylene output does not imply physiological irrelevance, as ethylene can act at very low concentrations as a modulatory signal, influencing tissue sensitivity to other hormones and fine-tuning ripening-related gene expression (Nakano et al. 2003; Wu et al. 2022). In this context, ABA accumulation in the flesh during 120–150 DAFB may contribute to priming ethylene responsiveness, whereas the subsequent decline in ABA and the late rise in JA, particularly in the apical region, may reflect a shift towards senescence-associated programmes that can occur with minimal ethylene production.

Collectively, these findings indicate that persimmon development and maturation are governed by the coordinated action of multiple plant hormones. The dynamic, tissue-specific changes observed across stages underscore the importance of temporal and spatial control of hormone biosynthesis and signalling. A precise balance among these pathways appears fundamental to orchestrate the transitions from growth to maturation and, ultimately, ripening.

Hormonal Role of the Calyx During Fruit Development

The pattern of phytohormone changes in detached calyx tissue differed markedly from that observed in whole fruit. As noted above, shifts in calyx ethylene and CO₂ production are closely linked to physicochemical processes during development and ripening. Notably, the peak in ethylene and CO₂ production at 120 DAFB coincided with the maximum accumulation of ABA and 7-OH-ABA in the same tissue. Previous studies indicate that ABA can act as an early positive signal triggering the climacteric ethylene cascade, with reciprocal feedback between ABA and ethylene pathways coordinating the progression of ripening (Wu et al. 2023). Mechanistically, ABA signaling can enhance ethylene biosynthesis by activating calcium-dependent protein kinases

(e.g., CPK4/11), which stabilise the ACS6 enzyme via phosphorylation, and by inducing ABA-responsive transcription factors such as *AREB/ABF*, that promote the expression of ethylene-biosynthetic and ripening-associated genes (Gupta et al. 2022; Wu et al. 2023). In the present study, the synchrony between the ABA peak in the calyx and the transient ethylene rise at 120 DAFB supports the hypothesis of localised hormonal crosstalk. Additionally, the increase in CO₂ production at this stage suggests enhanced metabolic activity, possibly linked to the onset of ripening.

In persimmon, the calyx serves as a protective structure during early fruit development. Previous work has shown that the persimmon calyx exhibits high levels of phytochemicals with antioxidant properties, arising from defence mechanisms against biotic stress (Lee et al. 2023; Santino et al. 2013). In both climacteric and non-climacteric fruit, JA is positively associated with stress responses, activating defence-related gene expression (Ali and Baek 2020; Santino et al. 2013). In the present study, in contrast to the pattern observed in the fruit flesh, JA levels were markedly high in the calyx during the initial developmental stages and declined progressively through maturation. A similar pattern has been reported for cape gooseberry, a fruit characterised by a large, protective calyx, where high JA concentrations in the calyx have been reported during early stages of fruit development, followed by a gradual decline throughout maturation (Alvarez-Florez et al. 2017). This decline has been linked to a reduction in the need for defence signalling as the fruit transitions from a developmentally sensitive phase, when susceptibility to biotic stress is greatest, towards later stages in which structural barriers and constitutive chemical defences predominate.

Besides, unlike the fruit flesh, the markedly higher ethylene and JA levels in the calyx detected in the present study, suggests that this structure may constitute a site where JA-associated senescence regulation intersect with ethylene signalling. This interaction could contribute to coordinating maturation, as JA signalling is known to frequently crosstalk with ethylene pathways during stress and senescence modulation (Kim et al. 2015; Pérez-Llorca et al. 2023).

Regarding CK, relatively few studies have examined their role in calyx development. In *Physalis floridana*, He and Saedler (2007) showed that exogenous CK induce calyx expansion, promoting formation of the characteristic inflated calyx. They concluded that cytokinin availability is essential for calyx cell elongation and proliferation, and in its absence, even with expression of cell-division genes such as *MPF2*, sepal cells remained small and unexpanded. In pear, Yu et al. (2025) reported differential hormonal regulation of calyx retention during flowering (0–10 DAFB). In plants with a persistent calyx, auxin levels were high, potentially maintaining cellular activity and preventing abscission; by

contrast, in genotypes exhibiting calyx abscission, CK levels in the calyx declined, suggesting a role for CK in inhibiting calyx senescence and abscission at early stages.

The relatively high CK levels detected in the calyx suggest that this organ may actively contribute to fruit development. CK are key regulators of source–sink relationships and nutrient allocation, and increased CK activity is commonly associated with enhanced sink strength and mobilization of resources to developing organs (McIntyre et al. 2021). In persimmon, the developmental relevance of the calyx is supported by calyx-lobe removal studies showing strong reductions in CO₂ exchange and inhibition of fruit growth, indicating that disruption of calyx function compromises physiological processes linked to fruit sink activity (Yonemori et al. 1995, 1996).

Conclusions

This work provides new information regarding the tissue-specific dynamics of abscisic acid, jasmonic acid and cytokinins in ‘Rojo Brillante’ persimmon, revealing how distinct hormonal dynamics in the apical flesh, basal flesh and calyx coordinate key developmental transitions, from early cell division and expansion through colour break, firmness loss and senescence. By mapping these spatial and temporal patterns, we have uncovered key regulatory events, such as the transition from cytokinin-driven growth to ABA-mediated on-tree ripening signalling, as well as the localized ABA–ethylene interplay in the calyx, that could be involved in the onset of fruit ripening. These insights provide a cohesive framework for understanding persimmon maturation that establishes the basis for future agronomic and postharvest research into the coordinated control of growth, colour break, softening, and senescence of persimmon. From an industry perspective, the identification of tissue-specific hormonal profiles may help refine harvest timing and support the development of targeted pre- and postharvest strategies, such as the optimized use of plant growth regulators or ripening-modulating treatments, to improve fruit quality and reduce postharvest losses.

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Author Contributions Nariane Q. Vilhena: data curation, formal analysis, investigation, validation, writing – original draft, writing – review and editing. M. Carmen Alamar: investigation, methodology, supervision, writing – review and editing, resources. Emma R. Collings: data curation, formal analysis, methodology, validation. Ana Moreno: data curation, formal analysis, investigation. Alejandra Salvador: conceptualization, funding acquisition, investigation, methodology, supervision, writing – review and editing, project administration, resources.

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Data Availability Data will be made available on request.

Declarations

Competing Interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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