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Reproductive biology, parthenocarpy, and fruit quality traits of the citrus hybrid 'Faustrime' (*Citrus australasica* × [*C. aurantifolia* × *Fortunella japonica*])

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Abstract

'Faustrime' is a hybrid between Australian finger lime, also known as caviar lime (*Citrus australasica*), and 'Eustis' limequat (*C. aurantiifolia* × *Fortunella japonica*), originally developed in California. Despite its growing commercial interest, this genotype has not yet been comprehensively characterised from morphological and agronomic perspectives. In the present study, we assessed flowering intensity, fruit set, parthenocarpic ability, and fruit development. Notably, 'Faustrime' requires a lower thermal accumulation to induce flowering through *CiFT3* gene expression than sweet orange (*Citrus sinensis* [L.] Osbeck), and it displays a marked capacity for summer flowering. Regarding fruit set, the hybrid achieves an optimal crop load due to its high parthenocarpic ability, which exceeds that of both sweet orange and its parental species, *C. australasica*. Consistent with this behaviour, 'Faustrime' synthesises higher levels of auxin and bioactive gibberellins (GA_1, GA_4, and GA_7) than its progenitors. In addition, this hybrid exhibits high nutritional value and distinctive organoleptic characteristics. Its pollen grains are pentacolpate, and both the ovary and fruit are oval in shape. The fruit contains small, spherical juice vesicles with a slightly acidic taste and a crunchy texture, which are highly appreciated in haute cuisine. Furthermore, the flavedo shows a high concentration of essential oils, mainly D-limonene (52%), γ-terpinene (12%), citronellal (15%), and δ-3-carene (5%), together with *n*-alkanes and coumarins, compounds valued in the pharmaceutical, perfumery, and cosmetic industries. Overall, these characteristics underline the potential of 'Faustrime' as a novel citrus genotype with agronomically relevant traits and prospective economic viability.

Keywords: Faustrime, *CiFT3*, Essential oils, Flowering, Fruit set, Gibberellins, Phenology

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Introduction

Australia's long geographic isolation and its diverse geological and climatic conditions have resulted in a distinctive biodiversity, with a considerable number of endemic plant species[1]. Consequently, Australian citrus species have evolved differently from other members of the genus *Citrus*, adapting to unusual soil conditions, salinity, and extreme drought[2]. Notably, Australia is home to three ancestral citrus taxa: *Microcitrus australis* (commonly known as round lime), *Microcitrus australasica* (finger lime), and *Eremocitrus glauca* (desert lime)[3].

This unique genetic diversity is currently being exploited in breeding programmes aimed at introducing tolerance to high temperatures and water stress, as well as improving resistance to diseases. For instance, Australian citrus species belonging to the genera *Microcitrus* and *Eremocitrus*, officially re-classified as *Citrus*, have recently been reported to exhibit tolerance or resistance to Huanglongbing (HLB), a devastating citrus disease for which no effective cure is currently available[4,5]. This resistance has been associated with the presence of a stable antimicrobial peptide that acts directly against the causal bacterium[6].

Beyond their remarkable adaptability to extreme environmental conditions and diseases, Australian citrus species also exhibit considerable potential for human health. In recent years, increasing interest in foods with antioxidant properties has stimulated studies on the nutritional value of finger lime fruit[7]. Phenolic compounds have been identified in the peel, pulp, and seeds of several finger lime species[7]. These compounds are known to neutralise free radicals, inhibit lipid peroxidation, and prevent oxidative damage, thereby potentially reducing the risk of cancer, diabetes, and cardiovascular diseases[8]. In addition, finger lime peel exhibits an intense floral–lime aroma and a distinctive organoleptic profile[9]. As a result, finger lime is valued both for its fresh fruit in haute cuisine and for its rind as a by-product in the perfumery industry.

Commercial cultivation of Australian finger lime began approximately 25 years ago, and currently, around 15 cultivars and hybrids are registered with the Australian Cultivar Registration Authority (ACRA). In the context of global climate change, finger lime cultivation is emerging as a promising alternative to conventional citrus crops due to its high resistance to diseases and its adaptability to extreme environmental conditions.

Although Lv et al.[10] evaluated several traits of nine *C. australasica* cultivars, including botanical characteristics, phenology, fruit morphology, and other descriptive attributes. Information regarding their physiological traits and agronomic performance remains limited. This knowledge gap is particularly evident for citrus hybrids. In the present study, we address this issue using the hybrid 'Faustrime', a cross between finger lime and the 'Eustis' limequat. The objectives of this work were to characterise its morphology, flowering phenology, fruit set, and mature fruit traits.

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Materials and methods

Plant material and experimental layout

The experiment was conducted in an experimental plantation comprising 'Faustrime' (*Citrus australasica* × [*C. aurantifolia* × *Fortunella japonica*]) finger lime, and two sweet orange (*C. sinensis*) cultivars, 'Pineapple', a seeded cultivar, and 'Lane late', a parthenocarpic cultivar. All trees were grafted onto *Citrus macrophylla* rootstock. The orchard was established on clay soil in Almussafes (València, Spain), with a planting density of 3.5 m × 5 m. Ten adult trees per genotype were randomly selected based on uniformity in canopy size and vigour. Irrigation, fertilization, and pest control were performed according to standard commercial practices.

Flowering intensity of 'Faustrime' was assessed in early spring and mid-summer and compared with that of 'Lane late', a non-alternate-bearing *C. sinensis* cultivar that flowers regularly each year. Two branches per tree, each bearing approximately 200 nodes, were selected on both the north- and south-facing sides of the canopy. The number of flowers per branch was recorded, and flowering intensity was expressed as flowers per 100 nodes to account for differences in branch size.

Fruit set was determined by selecting and labelling 400 flowers per tree on five trees, 15 d before anthesis. Of these, 200 flowers were left intact to obtain open-pollinated ovaries (OP), while the remaining 200 were emasculated 5 d before anthesis, when pollen was still immature, and subsequently bagged to prevent cross-pollination, thus generating unpollinated ovaries (UO). The same experimental procedure was applied to 'Pineapple' sweet orange, a cultivar widely used as a reference model in studies addressing fertilization versus parthenocarpy[11–13]. At harvest, the number of fruits from labelled flowers was recorded, and fruit set was expressed as a percentage.

To monitor fruit growth and development, ten fruits per tree from five 'Faustrime' trees were randomly selected and periodically measured for fruit length, equatorial diameter, fresh weight, and rind colour from early June to mid-January. At harvest, 40 homogeneous fruits from five trees were collected for essential oil extraction from the rind.

Fruit and leaf colour were evaluated using Hunter *a* and *b* coordinates. Three measurements were taken per fruit at the equatorial zone, and one measurement per leaf was taken from 25 fully expanded leaves at the central area of the adaxial surface. Colour measurements were performed using a CR-400 Chroma Meter (Konica Minolta Optics Inc., Osaka, Japan).

For *CiFT3* gene expression analysis, leaves from 'Faustrime' and 'Lane late' were sampled monthly from September to December. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

Sequence analysis

Gene sequences used for expression analysis were retrieved from the Phytozome v.13 database (www.phytozome.net). Sequence alignment and phylogenetic analysis (Supplementary Fig. S1) were performed using MEGA11 software (www.megasoftware.net). Sequences from the species most closely related to 'Faustrime', *C. australasica*, for which a reference genome is available, were obtained from the Citrus Pan-genome to Breeding Database (CPBD; www.citrusgenomedb.org).

Gene expression analysis

Flowering time was investigated through gene expression analysis of the FLOWERING LOCUS T gene (*CiFT3*). From late August to late December, one fully expanded mature leaf per tree was periodically collected from five 'Faustrime' and five 'Lane late' trees. Samples were immediately frozen in liquid nitrogen, transported to the laboratory, and stored at −80 °C until further analysis.

Total RNA was extracted from three frozen biological replicates per species and sampling date using the RNeasy Plant Mini Kit (Qiagen). RNA quality and integrity were assessed by a Nanodrop ND-1000 spectrophotometer, using the OD_{260}/OD_{280} ratio, and gel electrophoresis. cDNA was obtained from 1 μg total RNA using the PrimeScriptTM RT Reagent Kit (Perfect Real Time; TAKARA bio Europe) in a final reaction volume of 10 μL. Quantitative real-time PCR (qRT-PCR) was performed using a Rotor-Gene Q 5-Plex system (Qiagen, USA) and the TB Green® Premix Ex Taq™ II PCR Kit (Takara Bio Europe). Reaction mixtures and cycling conditions were set according to the manufacturer's instructions, with minor modifications. Each 25 μL reaction contained 2 μL of diluted cDNA, 12.5 μL of TB Green Premix Ex Taq II (2X), 1 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, and 8.5 μL of nuclease-free water. The amplification program consisted of an initial pre-incubation step at 95 °C for 15 min, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Primer sequences are listed in Supplementary Table S1.

Essential oil analysis

Oil extraction

Essential oil extraction was performed using Ultrasonic-Assisted Extraction (UAE). Samples were obtained by cold superficial scraping of the flavedo with a kitchen grater, carefully avoiding the albedo, until approximately 1 g of tissue was collected. The plant material was weighed and ground to facilitate essential oil release and subsequently subjected to an ultrasonic bath for 5 min at room temperature to enhance extraction efficiency[14,15]. After ultrasonic treatment, the mixture was filtered through glass wool using a syringe to remove solid residues. The resulting emulsion was transferred into Erlenmeyer flasks (25 mL), to which 2 mL of dichloromethane was added. The flasks were shaken for 15 min at 120 rpm and room temperature on an orbital shaker to ensure homogenization. The contents were then transferred to test tubes and centrifuged at 5,000 rpm for 10 min at 4 °C.

The organic phase was carefully collected using a Pasteur pipette, and anhydrous sodium sulphate was added until complete clarification of the extract. The solution was subsequently filtered using a Pasteur pipette packed with cotton wool and concentrated by solvent evaporation in a rotary evaporator. Evaporation was carried out at 25–30 °C under reduced pressure adjusted to allow gentle boiling. When solvent evaporation ceased, the flask was removed, sealed, and weighed after taring with the stopper in place. This procedure was repeated until a constant weight of essential oil was achieved. Finally, the purified extract was aliquoted into nine insert vials to obtain three essential oil samples, corresponding to three fruits per biological replicate. The vials were sealed with Parafilm and stored at 4 °C in the dark until further analysis.

Gas chromatography with flame ionization detection (GC–FID) and mass spectrometry (GC–MS)

Samples were analysed by gas chromatography coupled with flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). Quantitative analysis was performed using a

Clarus 500 gas chromatograph (PerkinElmer Inc., Wellesley, PA, USA) equipped with an FID detector and a ZB-5 capillary column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness; Phenomenex Inc., Torrance, CA, USA). The injection volume was 1 μL. The oven temperature program was set from 50 to 250 °C at a ramp rate of 3 °C min^{-1}. Helium was used as the carrier gas at a constant flow rate of 1.2 mL min^{-1}. Injector and detector temperatures were maintained at 250 °C. The relative composition of the essential oil was calculated as the percentage of individual peak areas relative to the total chromatographic area, without applying response correction factors, using TotalChrom 6.2 software (PerkinElmer Inc.).

Qualitative analysis was carried out using a Clarus GC–MS system (PerkinElmer Inc.) under the same chromatographic conditions described for GC–FID, including the capillary column and carrier gas. Electron impact ionization was applied at 70 eV, with the ion source temperature set at 200 °C. Mass spectra were acquired in total ion current (TIC) mode over an *m/z* range of 45–500. Data acquisition and processing of total ion chromatograms and mass spectra were performed using TurboMass 5.4 software (PerkinElmer Inc.). Retention indices were calculated by analysing a homologous series of *n*-alkanes (C_8–C_{25}; Supelco, Bellefonte, PA, USA) under identical chromatographic conditions.

Hormonal analysis

Endogenous concentrations of seven gibberellins (GAs) and indole-3-acetic acid (IAA) were quantified in pollinated ovaries of *C. australasica* and 'Faustrime', as well as in unpollinated ovaries of 'Faustrime'. Ovaries were collected after fruit set (BBCH stage 72;[16]), immediately frozen in liquid nitrogen, and ground to a fine powder.

Three replicates of five ovaries were ground into a fine powder. Approximately 50 mg of fresh or dry tissue was extracted with 80% methanol containing 1% (v/v) acetic acid. Deuterium-labelled hormones were added as internal standards, and samples were incubated at 4 °C for 1 h. Hormone extraction was performed following the protocol described by[17], with minor modifications. After desalting, extracts were purified using reversed-phase HLB cartridges, eluted with 80% methanol containing 1% acetic acid, and subsequently fractionated through MCX and WAX ion-exchange columns. The final residue was resuspended in 5% acetonitrile containing 1% acetic acid.

Hormone separation was achieved by reverse-phase ultra-high-performance liquid chromatography (UHPLC) using a 5%–50% acetonitrile gradient containing 0.05% acetic acid at a flow rate of 400 μL min^{-1} for 14 min. Quantification was carried out by selected ion monitoring (SIM) using a Q Exactive mass spectrometer. Deuterium-labelled GAs (GA_{19}, GA_{20}, GA_1, GA_{12}, GA_{24}, GA_4, and GA_7) were used as internal standards, and IAA calibration curves were generated using both labelled and unlabelled standards. Hormone concentrations were calculated using embedded calibration curves with Xcalibur 2.2 SP1 and TraceFinder software at the IBMCP-UPV hormone quantification facility.

Statistical analysis

Data were analysed using STATGRAPHICS Plus software. Analysis of variance (ANOVA) was performed, and means were separated using Fisher's least significant difference (LSD) test ($p < 0.05$).

Results and discussion

Morphology and phenology. Leaf, flower, and fruit development

'Faustrime' undergoes three flushing events per year under temperate climatic conditions. The first flush occurs in spring, approximately 30 d later than in most citrus species[18], followed by a second flush in early summer and a third flush in autumn, coinciding with declining summer temperatures (Fig. 1a). At budburst, the buds display a purplish hue at the apical region (Fig. 1a), a trait

Fig. 1 Three flush shoots of (a) 'Faustrime' finger lime showing leaves and axillary thorns. (b) Leaf detail showing the adaxial and abaxial surfaces, and (c) thorn shown in longitudinal view and (d) in axillary position.

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inherited from one of its parental genotypes, the limequat. Leaves are small, approximately 2.5 cm wide and 4.5 cm long, lanceolate, and with slightly serrated margins. During development, leaves progressively lignify and acquire a light green colour (a/b = 0.71), with the adaxial surface darker than the abaxial surface (Fig. 1b). Each node bears an axillary thorn measuring 1.5–3.0 cm in length (Fig. 1c, d), which persists beyond the juvenile stage.

Flower buds are nearly spherical during the early stages of development (BBCH55)[16] (Fig. 2a) and become ovoid at BBCH58. At anthesis (BBCH65), stamens exhibit a deep yellow colour and produce viable pollen. Pollen grains are pentacolpate (Fig. 2b, c), in contrast to the predominantly tetracolpate pollen of most citrus cultivars[19]. Following anthesis, stamens dehydrate, petals abscise, and fruit set is initiated. At this stage (BBCH69), the elongated ovary, a characteristic feature of this species, becomes exposed and is clearly separated from the calyx (Fig. 2d), unlike in other citrus species[20].

The fruit subsequently enters Phase I of development, characterised by cell division and lasting approximately one month, and is accompanied by an initial wave of physiological fruitlet drop (BBCH73). This stage is followed by Phase II, characterised by cell enlargement (Fig. 2e), during which fruit growth continues for approximately six months, reaching maximum size in October (BBCH81). Thereafter, Phase III begins, during which juice vesicles acquire their characteristic spherical shape and color (Fig. 2f, g), the fruit undergoes color change, reaches optimal harvest maturity (BBCH 89; Fig. 2a), and subsequently enters senescence. During this final stage, juice vesicles acquire their characteristic spherical shape and colour (Fig. 2f, g), and the fruit reaches optimal harvest maturity. Notably, the distinctive vesicle shape and size, soft green colour, aroma, delicately acidic flavour, and crisp texture confer high culinary value, making the fruit particularly attractive for refined gastronomic applications.

Flowering

Flowering capacity among finger lime species is heterogeneous, with most species flowering in spring and some exhibiting a tendency towards alternate bearing[21]. In the Northern Hemisphere under Mediterranean climatic conditions, 'Faustrime' flowers in spring (April) and again in mid-summer (July) (Fig. 3), as do *Fortunella* spp.[22,23]. This flowering behaviour contrasts with that of most finger lime varieties, which typically flower only once, in spring[10]. Spring flowering in 'Faustrime' is quantitatively greater (33 flowers per 100 nodes) than summer flowering (19 flowers per 100 nodes) but remains substantially lower than that observed in 'Lane late' sweet orange (78 flowers per 100 nodes).

Out-of-season summer flowering of citrus under Mediterranean climate conditions is also common in lemon, a species closely related to the parental species of 'Faustrime', and it can even be induced in summer by a period of water stress to produce off-season fruit that ripens in about one year, a technique known as 'forzatura'[24]. Similarly, fruits of 'Faustrime' produced from summer flowering mature in May–July of the following year and show fruit quality parameters comparable to those obtained from spring flowering, including fruit size, color, total soluble solids, and titratable acidity (data not shown).

To determine the timing of floral induction, the expression of the flowering integrator gene *CiFT3*[25,26] was analysed during summer and autumn. In 'Lane late', *CiFT3* expression increased significantly during October (Fig. 4a), reaching a maximum in late November, coinciding with the seasonal decrease in temperature (Fig. 4b). In contrast, 'Faustrime' exhibited an earlier increase in *CiFT3* expression, beginning as early as September and reaching relative expression levels higher than those observed in 'Lane late'. Expression levels subsequently declined gradually, at least until the end of December (Fig. 4b). These results indicate that this finger lime

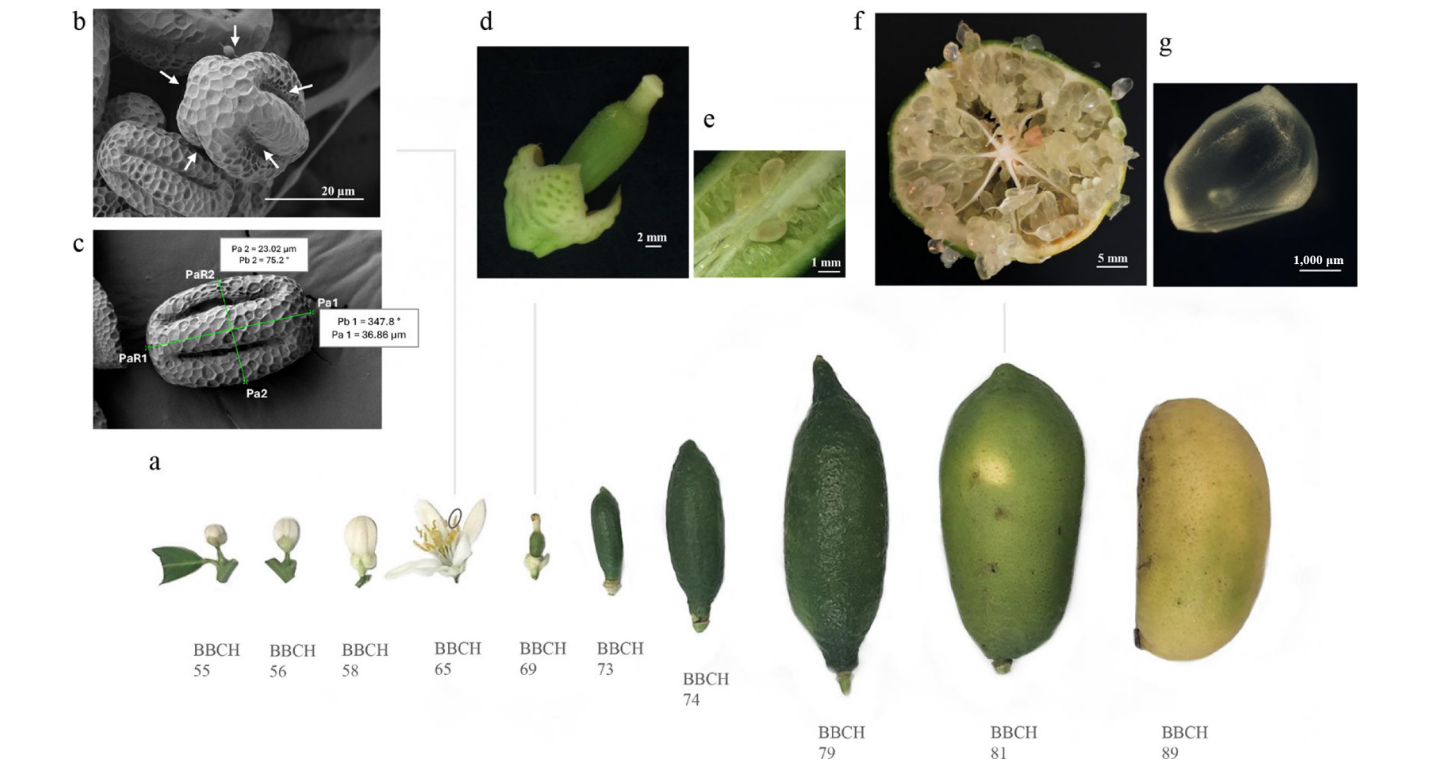

Fig. 2 Phenological stages of (a) flower and fruit development, (b), (c) pollen grains, (d) fruitlet, (e) juice vesicle cell enlargement, and (f), (g) juice vesicles of 'Faustrime' finger lime.

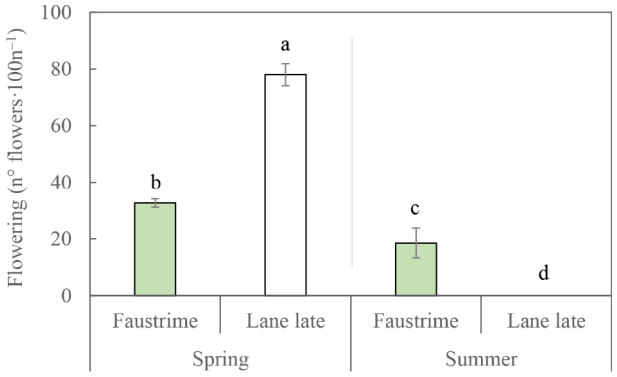

Fig. 3 Spring and summer flowering intensity of 'Faustrime' finger lime and 'Lane Late' sweet orange. Values are the mean of two branches per tree and ten trees. Different letters indicate significant differences ($p < 0.05$).

hybrid has lower thermal requirements for floral induction than sweet orange cultivars[23,27], leading to the expression of *CiFT3* gene in leaves approximately one month earlier (Fig. 4a).

Given that 'Faustrime' is a hybrid involving Mexican lime (*C. aurantifolia*), a species that under tropical conditions initiates flowering in response to a dry season[28–30], it is not surprising that, under Mediterranean Basin conditions, 'Faustrime' also exhibits a summer flowering event.

Fruit set

As occurs with its parental species *C. aurantifolia*[31,32] and *C. australasica*[33], 'Faustrime' exhibits cross-pollination with kumquat (*Fortunella* spp.), Australian finger lime (*C. australasica*), Persian lime (*C. latifolia*), and, to a lesser extent, Clementine mandarin (*C. clementina* Hort. ex Tanaka) and lemon (*C. limon*). In contrast, sweet orange (*C. sinensis*) and sour orange (*C. aurantium*) scarcely fertilised 'Faustrime'. In our experiment, 30 d after anthesis, approximately 25% of naturally pollinated 'Faustrime' flowers successfully set fruit, whereas 18% of unpollinated flowers also set fruit, demonstrating a substantial parthenocarpic ability. These results contrast sharply with those obtained for the sweet orange cultivar 'Pineapple', which is entirely dependent on fertilisation[11]. In this cultivar, fruit set in pollinated flowers barely exceeded 10%, and unpollinated flowers failed to set fruit altogether (Fig. 5a), as previously reported by Ben-Cheikh et al. and Bermejo et al. [11,13].

At harvest, fruits derived from open-pollinated 'Faustrime' flowers contained, on average, 9.2 seeds per fruit, whereas parthenocarpic fruits had fewer than one seed, averaging 0.6 seeds per fruit (Fig. 5b); these differences were statistically significant ($p < 0.05$). Notably, fruit size did not differ significantly between pollinated and parthenocarpic fruits (Fig. 5c), despite previous reports describing a positive correlation between seed number and final fruit size in sweet orange[34], grapefruit[35], and mandarin cultivars[36].

In *Citrus*, high parthenocarpic ability is closely associated with auxin and gibberellin biosynthesis in the ovary wall[12]. A comparison between hormone contents in pollinated ovaries of 'Faustrime' and those of its direct parental species, *C. australasica*, revealed a greater ability of the hybrid to synthesise bioactive gibberellins. Specifically, concentrations of GA_4 (1.61 ng g^{-1}) and GA_1 (2.03 ng g^{-1}) in 'Faustrime' ovaries were approximately fivefold higher than those measured in *C. australasica* (GA_4: 0.28 ng g^{-1}; GA_1: 0.38 ng g^{-1}) (Fig. 6c, g). Consistent differences were also observed for GA_7, with concentrations in 'Faustrime' (1.92 ng g^{-1}) tenfold higher than those in the parental species (0.18 ng g^{-1}) (Fig. 6d). In parallel, indole-3-acetic acid (IAA) concentrations were approximately sixfold higher in 'Faustrime' (26.7 ng g^{-1}) than in *C. australasica* (4.70 ng g^{-1}) (Fig. 6h).

A similar pattern was observed in unpollinated ovaries of 'Faustrime', which consistently exhibited significantly higher concentrations of bioactive gibberellins and IAA than those of *C. australasica* (Fig. 6). Collectively, these results indicate that auxin and gibberellin biosynthetic pathways are more active in 'Faustrime', irrespective of the pollination status of the ovary, thereby highlighting the strong parthenocarpic ability of this hybrid.

It should be noted that gibberellin analyses were performed 10–12 d after anthesis (BBCH stage 72), a developmental stage at which fruits displaying obligate and facultative parthenocarpy share a second upregulation of gibberellin biosynthesis following *CYCA1.1* induction[12].

Fruit characteristics

Mature 'Faustrime' fruits reach approximately 3 cm in diameter and 8 cm in length, with an average fresh weight of 45 g (Fig. 7a–c). Colour change occurs in late October, coinciding with the completion of fruit growth (Fig. 7d) and the onset of lower temperatures (Fig. 4b). As the optimal levels of total soluble solids and titratable

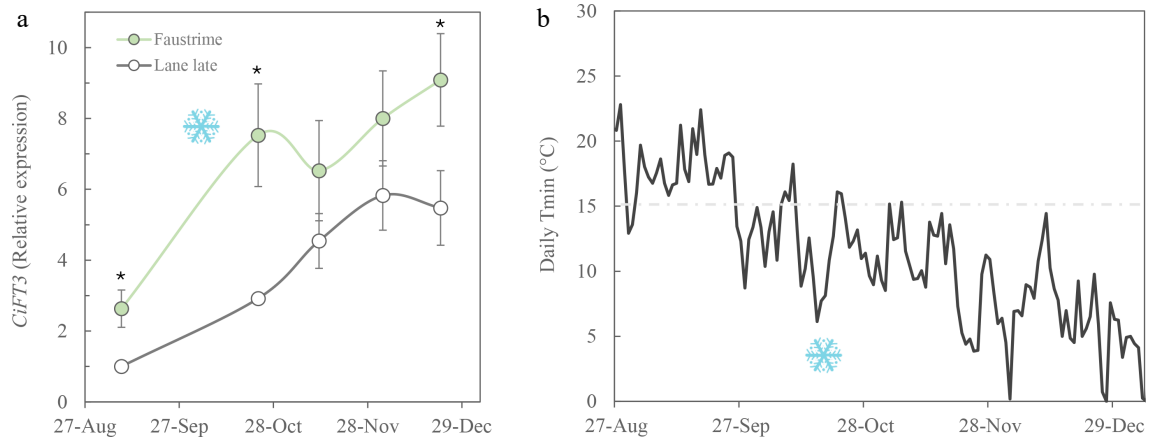

Fig. 4 (a) Time course of *CiFT3* expression in leaves of 'Faustrime' finger lime and 'Lane Late' sweet orange from late August to December, and (b) daily mean temperature. Data are the means ± SE of three independent replicates ($n = 3$). Asterisks indicate significant differences ($p < 0.05$) for each sampling date. In some cases, SE bars are smaller than the symbol size.

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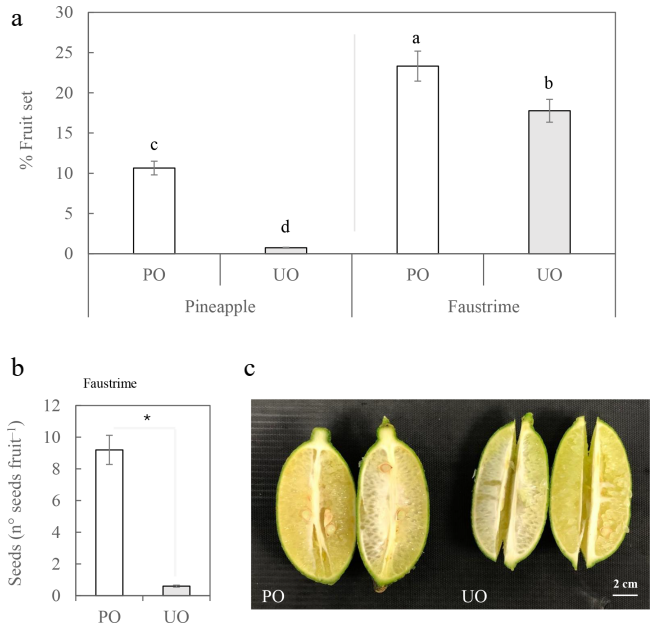

Fig. 5 (a) Fruit set in pollinated (PO) and unpollinated (UO) flowers of sweet orange 'Pineapple' and finger lime 'Faustrime', (b) number of seeds per fruit of 'Faustrime', and (c) final fruit size at harvest. Fruit set values are means ± SE based on 400 flowers per tree and five trees. Seed number per fruit represents means ± SE of five PO and UO fruits per tree and five trees. Different letters or asterisks indicate significant differences ($p < 0.05$).

acidity are reached one month before color break (early October; Fig. 7e, f), and mature fruits do not undergo preharvest fruit drop, the harvesting period extends from early September until April. Over a six-year period, the experimental plantation produced an average yield of 2,600 kg ha^{-1}.

Although the fruit is predominantly consumed fresh, the chemical composition of the peel, which is rich in essential oils, suggests considerable potential for applications in the pharmaceutical, perfumery, and cosmetic industries. *D*-Limonene is the predominant component, accounting for 43% of the total peel essential oils (Fig. 8a). This compound exhibits strong antioxidant and anti-inflammatory properties[37]. In commercially important citrus species such as mandarin, orange, and grapefruit, *D*-limonene concentrations are markedly higher, typically ranging from 85% to 95% of total peel essential oils[38]. In contrast, several minor compounds are present at higher proportions in 'Faustrime'. Citronellal, a compound with insect-repellent, antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant properties, generally does not exceed 1% in lemon peel oil[39], whereas in 'Faustrime' it reaches 15.2% (Fig. 8a). Other compounds present at relatively high concentrations include γ-terpinene, a molecule with strong antioxidant capacity[40], which accounts for 10.1%, and δ-3-carene, an antioxidant and anti-inflammatory compound, representing approximately 5%.

In addition, 'Faustrime' exhibited comparatively high concentrations of several citrus compounds of industrial relevance, including piperitone (2.7%) and linalool (2.6%), both known for their

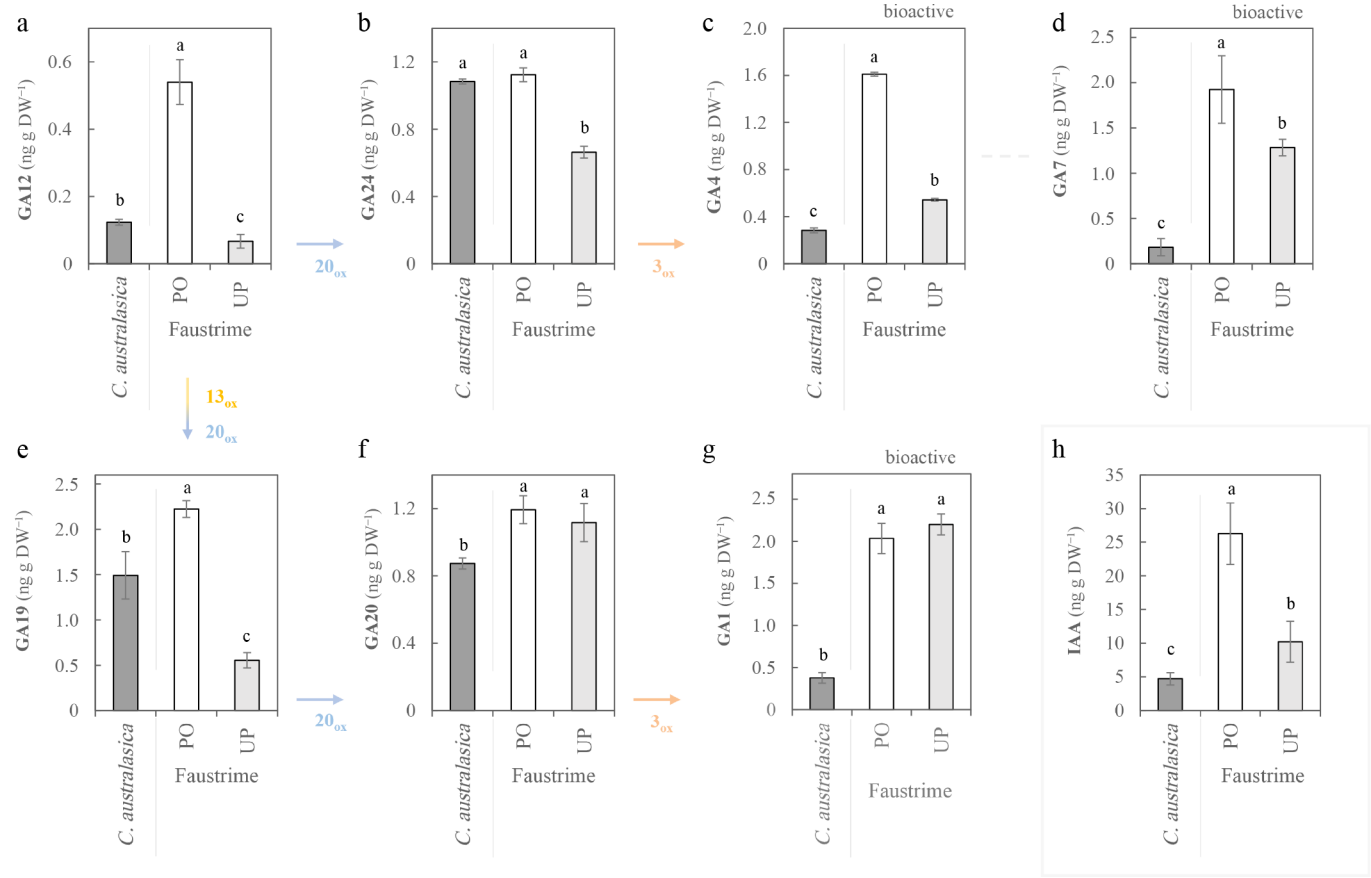

Fig. 6 (a)–(d) Endogenous concentrations of gibberellins from the non-hydroxylation pathway, and (e)–(g) the 13-hydroxylation pathway, and (h) auxin indole-3-acetic acid (IAA) contents in pollinated ovaries of *C. australasica* and 'Faustrime', as well as in unpollinated ovaries of 'Faustrime' at fruit set (BBCH stage 72). Arrows indicate the gibberellin biosynthetic enzymes GA13-oxidase, GA20-oxidase, and GA3-oxidase. Data represent the mean of three biological replicates, each consisting of five ovaries. Different letters indicate significant differences ($p < 0.05$).

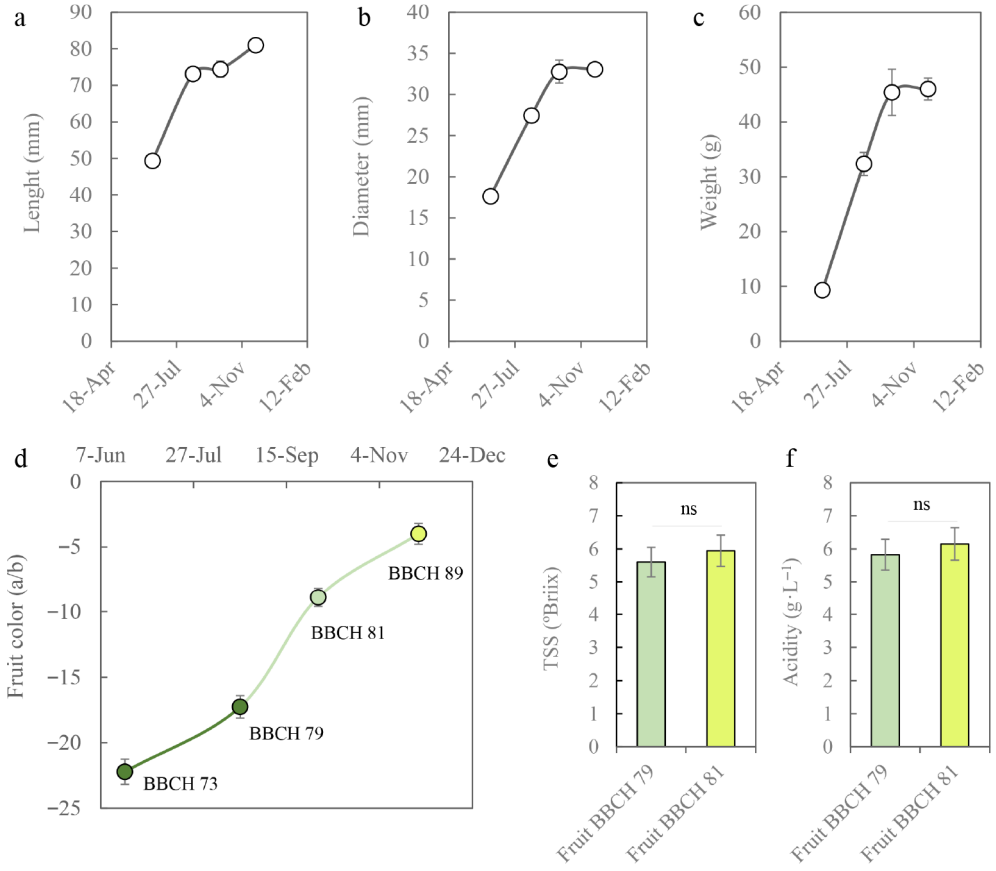

Fig. 7 Time course of 'Faustrime' finger lime development: (a) length, (b) width, (c) weight, (d) rind colour, (e) soluble solids, and (f) titratable acidity. Data are the means ± SE of ten fruits per tree and five trees. In some cases, SE bars are smaller than the symbol size.

a

Major constituents			
Compound	**%**	**Compound**	**%**
D-limonene	43	p-cymene	0.3
citronellal	15.2	neral	0.3
γ-terpinene	10.1	Germacrene-D	0.3
δ-3-carene	5.2	*cis*-pinene hydrate	0.2
piperitone	2.7	*cis*-*β*-ocimene	0.2
α-pinene	2.7	8-methoxypsoralen	0.2
linalool	2.6	b-caryophyllene	0.2
tetracosane	2.4	*α*-humulene	0.2
citronellol	1.5	*α*-terpinene	0.2
α-terpineol	1.3	docosane	0.2
myrcene	1.3	cryptone	0.1
linalool propanoate	1.3	hexadecanoic acid	0.1
isomenthone	1.1	octacosane	0.1
trans-piperitol acetate	1.1	*trans*-sabinene hydrate	0.1
sabinene	1	pentacosane	0.1
β-pinene	0.9	heptacosane	0.1
(E,E)-*α*-farnesene	0.7	citronellyl acetate	0.1
terpinolene	0.5	geranyl acetate	0.1
trans-*α*-bergamotene	0.5	pentadecane	0.1
α-thujene	0.4	decanal	0.1
geranial	0.4	tricosane	0.04
trans-piperitol	0.4	hexacosane	0.04
α-phellandrene	0.3		

b

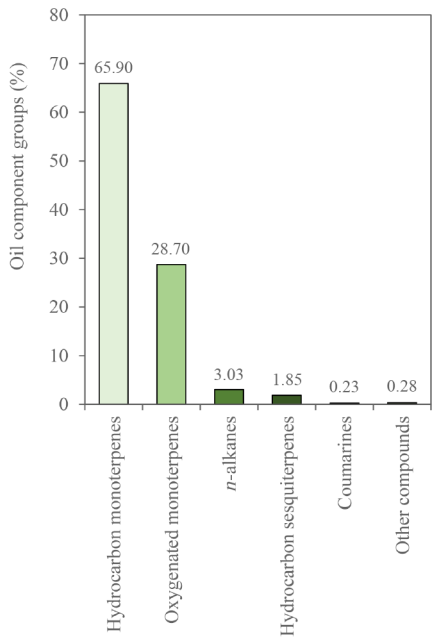

Fig. 8 (a) Essential oil composition of 'Faustrime' finger lime, and (b) their chemical class classification. Data are the means of 400 fruits from five trees.

antiseptic and soothing properties[41]; *α*-pinene (2.7%) and *β*-pinene (0.9%), which display well-documented antibacterial activity[42]; and *α*-terpineol (1.3%), a compound with multiple bioactive properties, including antioxidant and analgesic effects[43] (Fig. 8a). These results are consistent with those reported by[44], obtained using manual cold-pressure extraction of the rind.

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Classification of the identified compounds according to their chemical classes (Fig. 8b) revealed a distinctive profile compared with other citrus species[45,38]. Our results partially agree with those of Cozzolino et al.[46], who also reported that 'Faustrime' differs from other finger limes due to its high content of hydrocarbon monoterpenes (65.9%) and the presence of compounds belonging to the *n*-alkane and coumarin classes.

Conclusions

In conclusion, 'Faustrime' is a finger lime hybrid with considerable agronomic and commercial potential. Its flowering induction period is comparable to that of widely cultivated citrus species; however, it exhibits lower thermal requirements and is also capable of flowering during summer. The hybrid displays a high parthenocarpic ability, associated with enhanced biosynthesis of auxin and the bioactive gibberellins GA_1, GA_4, and GA_7. The extended harvesting period, which lasts until mid-spring, further enhances its agronomic value. Moreover, the small, spherical juice vesicles, delicately acidic flavour, and crisp texture contribute to its appeal for gourmet culinary applications. In addition, the distinctive chemical profile of the peel suggests potential uses in high-value sectors such as the pharmaceutical, perfumery, and cosmetic industries. Collectively, these traits position 'Faustrime' as an innovative citrus hybrid with diversified applications beyond fresh fruit consumption.

Author contributions

The authors confirm their contributions to the paper as follows: study conception and design, draft manuscript preparation: Marzal A, Mesejo C, Agustí M; methodology: Martínez-Fuentes A, Olivares-Fuster O, Reig C, Llorens-Molina JA; investigation: Marzal A, Martínez-Fuentes A, Reig C, Llorens-Molina JA; writing – review and editing: Marzal A, Agustí M. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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Conflict of interest

The authors declare the absence of any competing financial interests or personal relationships that could be perceived as influencing the work reported herein.

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