‘Jinju’ Pomelo Pollen Enhances Fruit Set and Development in Citrus maxima ‘Tomentosa’: Physiological and Proteomic Insights

Yaoxin Liu
Guangxi Academy of Agricultural Sciences
Huijun Qiu
Guangxi Academy of Agricultural Sciences
Weilun Xu
College of Plant Protection, South China Agricultural University
Xiaofan Zhou
College of Plant Protection, South China Agricultural University
Zhitao Ou
Guangxi Academy of Agricultural Sciences
Xi Sun
Yulin Forestry Research Institute
Juan Lu
Yulin Forestry Research Institute
Jieyu Li
Guangxi Academy of Agricultural Sciences
Xiangling Chen
Guangxi Academy of Agricultural Sciences
Changqing Chang
changcq@scau.edu.cn

College of Plant Protection, South China Agricultural University

Research Article

Keywords: Citrus maxima ‘Tomentosa’, ‘Jinju’ Pomelo, Cross-pollination, Fruit set, Endogenous hormones, Proteomics, Self-incompatibility

Posted Date: February 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8858104/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on July 6th, 2026. See the published version at https://doi.org/10.1186/s12870-026-09419-4.

Abstract

Citrus maxima ‘Tomentosa’, (commonly known as ‘Luchuan Juhong’) is a valuable medicinal and edible germplasm resource in China, renowned for its unique pharmacological properties. However, its commercial cultivation is hindered by strong self-incompatibility, leading to poor fruit set and significant physiological fruit drop. While artificial cross-pollination can mitigate this issue, the effectiveness of different pollen donors and the underlying molecular mechanisms remain poorly understood. In this study, we assessed four pomelo cultivars as pollen donors and identified ‘Jinju’ Pomelo to be the most effective, achieving fruit set rates of 78.00% (7 days post-pollination) and 44.00% (8 weeks post-pollination)—significantly higher than self-pollination (42.67% and 8.0%) or pollination with the commonly used ‘Hongrou’ pomelo (66.67% and 26.67%). Physiological analysis revealed that ‘Jinju’ Pomelo pollen had superior viability, with the shortest germination time (6.67 h) and the highest germination rate (89.68%), enabling rapid pollen tube growth before stigma senescence. Cross-pollination also triggered dynamic changes in endogenous hormone, characterized by a synergistic increase in auxin (IAA) and zeatin (ZT) alongside a sharp decline in abscisic acid (ABA) during the critical fruit-set window (5–7 days post-pollination). Quantitative proteomics further identified 367 differentially expressed proteins (DEPs) in cross-pollinated ovaries, enriched in pathways related to pollen recognition, pollen tube guidance, and phenylpropanoid biosynthesis. These findings suggest that compatible pollination triggers a coordinated hormonal and proteomic response, reducing abscission and enhancing fruit development. This study provides scientific insights for optimizing pollination strategies and supports the sustainable cultivation of *C. maxima* ‘Tomentosa’.

Introduction

Citrus maxima ‘Tomentosa’, widely designated by its local appellation ‘Luchuan Juhong’, represents a cornerstone of traditional medicinal and edible germplasm within the genus Citrus (Rutaceae). As a recognized Geographical Indication Product in China [1], it possesses substantial cultural heritage and economic significance, particularly in the Guangxi province. The fruit is chemically distinguished for its elevated concentrations of bioactive phytochemicals, notably total flavonoids and naringin. These compounds substantiate its long-standing application in traditional medicine as a potent antitussive and expectorant agent [1]. In contemporary pharmacological research, *C. maxima* ‘Tomentosa’ has attracted renewed attention for its potential utility as an adjunctive therapeutic intervention for respiratory viral infections, including COVID-19 [2], thereby augmenting market interest and demand. Despite its considerable economic potential and medicinal value, the Juhong industry encounters a critical biological impediment: severe physiological fruit abscission and consistently low natural fruit set rates. These reproductive challenges are primarily ascribed to gametophytic self-incompatibility, a genetic mechanism precluding self-fertilization [3–5], which results in agronomic yields that frequently fail to satisfy the escalating market demands [3–6].

The phenomenon of self-incompatibility in *Citrus* often leads to the arrest of pollen tube growth within the style, preventing fertilization and triggering the premature senescence of the flower [4, 6, 7]. To

circumvent the limitations imposed by this mechanism, the implementation of cross-pollination utilizing genetically compatible pollen donors has emerged as an essential agronomic practice [8–10]. Currently, 'Hongrou' pomelo serves as the predominant pollinator in commercial production systems. However, its efficacy exhibits significant variability under diverse environmental conditions, suggesting it may not be the optimal physiological match. Furthermore, the specific physiological and molecular mechanisms governing successful fruit set in *C. maxima* 'Tomentosa'—from the initial pollen-stigma interaction to the downstream hormonal signaling [8, 9]—remain insufficiently characterized. Optimizing yield necessitates the identification of a pollinator that synchronizes precisely with the physiology of the maternal plant to maximize fertilization efficiency [5, 7, 8].

The transition from a fertilized flower to a developing fruit—termed "fruit set"—is a complex developmental phase transition rigorously regulated by a dynamic network of phytohormones [11–13]. Auxin (IAA) and gibberellins (GA) function as central regulators in this progression, acting synergistically to inhibit the formation of the abscission zone and drive the initial phase of ovarian expansion [14–17]. Specifically, IAA plays a dual regulatory role: it directly suppresses the expression of genes associated with embryo abortion [17] and modulates GA metabolism to promote the accumulation of bioactive GAs in the nascent fruit [18–20]. Conversely, GA integrates various metabolic pathways, rewiring central metabolism to supply the requisite energy and carbon skeletons (sugars) essential for rapid fruit development [21–24]. The delicate equilibrium between growth-promoting hormones (IAA, GA, and Cytokinins) and growth-inhibiting hormones (e.g., Abscisic Acid, ABA) constitutes the primary determinant of fruit fate [25–27]. Elevated ratios of IAA/GA to ABA are generally regarded as conducive to fruit retention, whereas increased ABA levels frequently precipitate the transcriptional activation of cell wall-degrading enzymes, leading to the formation of the abscission zone [28–31]. Furthermore, recent studies in crops such as tropical pumpkin (*Cucurbita moschata* L.) and sweet cherry indicate that the precise manipulation of these hormonal balances, even exogenously, can significantly influence the success of fruit set [32–36].

Beyond hormonal regulation, successful fertilization relies upon precise, rapid, and compatible pollen-stigma interactions. This process involves a sophisticated cascade of molecular events, wherein proteins such as chemocyanin and stigma-specific cysteine-rich (SC) proteins facilitate pollen adhesion, hydration, and the subsequent directional guidance of the pollen tube through the stylar transmitting tissue [23]. In self-incompatible species, receptor complexes (e.g., the FER/ANJ/HERK1 complex in *Arabidopsis*) function as checkpoint mechanisms to recognize and reject "self " pollen while permitting compatible pollen tubes to reach the ovule [37, 38]. Elucidating how compatible pollen effectively circumvents these barriers to trigger the downstream hormonal cascades necessary for fruit set is paramount to the development of high-yield cultivation strategies.

In the present study, we systematically compared the efficacy of four distinct pomelo cultivars as pollen donors for *C. maxima* 'Tomentosa' to identify an elite pollinator. We determined that 'Jinju' Pomelo pollen significantly outperformed the widely utilized 'Hongrou' pomelo and other tested varieties. We further investigated the underlying biological mechanisms by integrating multiple levels of analysis:

characterizing pollen germination kinetics, tracking pollen tube growth dynamics via fluorescence microscopy, quantifying temporal shifts in endogenous hormones, and profiling proteomic alterations in developing ovaries. Our findings elucidate the physiological and molecular basis of improved fruit set, offering a robust theoretical framework for optimizing cultivation practices and ensuring the sustainability of the ‘Juhong’ industry.

Materials and Methods

Plant Materials

The experiment was conducted over three consecutive growing seasons (2022 to 2024) at the Lvfeng ‘Juhong’ Cooperative in Luchuan County, Guangxi, China. The orchard is situated in a typical subtropical monsoon climate zone characterized by red soil rich in organic matter, which is considered optimal for citrus cultivation. The experimental site received standard agronomic management, including uniform irrigation, fertilization, and pest control, to minimize environmental variability and ensure that observed differences were due to treatment effects. *C. maxima* ‘Tomentosa’ trees were planted at a spacing of 4 m × 6 m. Pollen donors comprised four distinct cultivars selected for their varied genetic backgrounds: ‘Sanhong’ pomelo, ‘Jinju’ Pomelo, ‘Majia’ pomelo, and ‘Hongrou’ pomelo. All pollen donor trees were cultivated at the Pomelo Resource Nursery of the Horticulture Institute, Guangxi Academy of Agricultural Sciences.

Pollen Collection and Storage

To ensure high pollen viability, collection was performed during the peak flowering season. Approximately 80 “balloon stage” flower buds (the developmental stage immediately preceding petal opening) were selected from the outer canopy of healthy donor trees on sunny mornings. This timing avoids moisture contamination from dew and ensures peak physiological activity. Anthers were carefully excised, wrapped in sulfuric acid paper to allow for aeration and prevent mold growth, and placed in a desiccator over silica gel. Once the anthers dehisced and pollen was released, it was collected, sealed in cryovials, and stored at -20°C until utilized for artificial pollination to maintain enzymatic activity.

Field Pollination Experiments

Five distinct pollination treatments were established to evaluate compatibility and efficacy in a randomized block design: natural pollination (control): Flowers were exposed to ambient pollination vectors (wind and local insect populations) without anthropogenic interference, serving as a baseline for natural fruit set rates. self-pollination: *C. maxima* ‘Tomentosa’ flowers were emasculated prior to anthesis and manually pollinated with their own pollen to rigorously assess the degree of self-incompatibility. cross-pollination (4 groups): *C. maxima* ‘Tomentosa’ flowers were emasculated and

manually pollinated with pollen derived from ‘Jinju’ pomelo, ‘Majia’ pomelo, ‘Sanhong’ pomelo or ‘Hongrou’ pomelo.

For each treatment, 25 inflorescences across three representative trees were selected. To standardize sink strength, two robust buds per inflorescence were retained, while others were removed. These buds were emasculated, pollinated, and immediately bagged with moisture-permeable paper bags to prevent contamination by foreign pollen. Bags were removed 7 days post-pollination (DPP) to allow for normal fruit development. Fruit retention and developmental metrics were monitored weekly until harvest.

Pollen Germination Assay

Fresh pollen was collected as described above. Pollen grains were distributed onto a standardized solid germination medium (containing sucrose, boric acid, and calcium nitrate optimized for Citrus pollen) and incubated at 28 ± 2°C in the absence of light to simulate the stylar environment. Germination progress was monitored microscopically at regular intervals. A pollen grain was technically defined as “germinated” when the length of the protruding pollen tube exceeded the diameter of the pollen grain itself. The germination rate (%) was calculated following a 12-hour incubation period by counting at least 100 grains per replicate across three biological replicates.

Pollen Tube Growth Observation

Pistils were harvested 7 days after pollination, a critical timepoint for fertilization where the pollen tube should have reached the ovary. Samples were fixed in FAA solution (Formalin-Acetic Acid-Alcohol), softened in 8 M NaOH for 12 hours to macerate the tissue, and stained with 0.1% aniline blue. Aniline blue specifically binds to callose in the pollen tube walls, allowing visualization under UV light. Pollen tube growth behavior and the depth of penetration within the style were visualized using fluorescence microscopy.

Quantification of Endogenous Hormones

Young fruit samples were collected at 1, 3, 5, and 7 DPP to capture the early, transient developmental signals that determine fruit fate. Samples were immediately flash-frozen in liquid nitrogen to halt metabolic activity and ground to a fine powder. Endogenous hormones (IAA, Zeatin [ZT], ABA) were extracted using a solvent mixture (2-propanol/H2O/concentrated HCl) and purified via solid-phase extraction. Quantification was performed using High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) on an AB Sciex QTRAP 6500 system (Nanjing Cavens Detection Technology Co., Ltd., Nanjing, China). Isotopically labeled internal standards were used to ensure quantification accuracy.

Proteomic Analysis

Ovaries from unpollinated flowers (Control) and Jinju Pomelo-pollinated flowers (7 DPP) were collected, washed with cold PBS to remove surface contaminants, and flash-frozen. Total protein was extracted using a lysis buffer containing protease inhibitors, followed by trypsin digestion. Peptides were labeled using Tandem Mass Tags (TMT) to allow for multiplexed quantification. The labeled peptides were fractionated and analyzed via LC-MS/MS. Data were processed to identify differentially expressed proteins (DEPs) using a rigorous threshold of $|\log2FC| \geq 0.58$ and $p < 0.05$. Gene Ontology (GO) and KEGG pathway enrichment analyses were performed to identify functional alterations in biological processes and metabolic networks.

Statistical Analysis

Fruit set rate was calculated as: (Number of fruits retained / Initial number of pollinated flowers) × 100. Branch fruit retention rate was calculated as: (Number of branches with fruit / Total branches) × 100. Data were analyzed using SigmaPlot 10.0 software. Differences between treatments were assessed using One-Way ANOVA followed by Duncan's New Multiple Range Test for significance, with $p < 0.05$ considered statistically significant.

Results

'Jinju' Pomelo Pollen Significantly Improves Fruit Set and Development

A comprehensive comparison of pollination strategies over three consecutive years revealed that cross-pollination significantly and consistently improved fruit set relative to self-pollination (42.67%), providing empirical confirmation of the self-incompatibility barriers inherent to *C. maxima* 'Tomentosa'. Among the array of tested donors, 'Jinju' Pomelo demonstrated superior performance, yielding the highest initial fruit set (78.00%). This performance significantly exceeded that of 'Sanhong' (70.67%), 'Red-fleshed' (62.00%), and 'Majia' pomelo (60.00%) (Fig. 1A), establishing 'Jinju' Pomelo as the most effective pollen source.

To assess the long-term stability of these findings, we monitored fruit retention metrics up to 8 weeks post-pollination. Although physiological fruit drop is a natural regulatory process in citrus, 'Jinju' Pomelo pollination maintained a robust final fruit set of 44.00%. This represents a striking 5.5-fold increase over the natural control (8.00%) and is significantly higher than the rate achieved by the commonly utilized 'Hongrou' pomelo (26.67%) (Fig. S1). To account for variability in branch vigor and resource allocation, we also calculated the "effective fruiting branch rate", which remained highest for 'Jinju' Pomelo (86.67%) (Fig. 1B). The results of continuous survey and follow-up at the fifth, sixth, seventh and eighth weeks after pollination showed that 'Jinju' Pomelo was the highest (75.33%), while that of the control group was only 14.67%, indicating that the overall trend of branch fruit retention rate and fruit set rate was consistent (Fig. S2).

Furthermore, detailed morphometric analysis indicated that fruits resulting from 'Jinju' Pomelo pollination exhibited significantly larger vertical and transverse diameters throughout the developmental

period (Fig. 2). This suggests that this specific cross-pollination event not only rescues fruit from abscission but also stimulates vigorous cell division and expansion, leading to superior fruit quality.

Superior Germination Characteristics of ‘Jinju’ Pomelo Pollen

Pollen viability assays revealed significant physiological disparities among the donor cultivars, which likely underpin the observed differences in field performance. ‘Jinju’ Pomelo pollen exhibited the most rapid activation kinetics, displaying the shortest germination latency (6.67 h) and the highest overall germination rate (89.68%) (Fig. 3). In stark contrast, *C. maxima* ‘Tomentosa’ self-pollen was notably sluggish, requiring 21.00 h to initiate germination and achieving a final rate of only 41.13%. The pollen from 'Red-fleshed' and ‘Majia’ pomelo cultivars demonstrated intermediate traits, with lower germination rates and extended lag times compared to ‘Jinju’ Pomelo. The rapid germination and high viability of ‘Jinju’ Pomelo pollen likely confer a decisive competitive advantage, ensuring that pollen tubes can initiate growth and reach the ovule prior to the onset of stigmatic senescence or the degradation of the embryo sac.

Cross-Pollination Overcomes Self-Incompatibility Barriers

Fluorescence microscopy of styles at 7 DPP provided direct, visual evidence of the compatibility mechanisms at play. In self-pollinated styles, pollen tube growth was severely restricted; the majority of tubes were arrested at the stigma surface or within the upper third of the style, often exhibiting swollen tips characteristic of self-incompatibility rejection responses. Conversely, styles pollinated with ‘Jinju’ Pomelo displayed a dense, unobstructed network of pollen tubes that successfully penetrated the full length of the transmitting tissue and reached the ovary base (Fig. 4). This unobstructed growth indicates highly compatible pollen-pistil signaling and the absence of S-RNase-mediated inhibition, which is a prerequisite for successful fertilization and subsequent seed set.

Cross-Pollination Reprograms Endogenous Hormone Balance

To elucidate the physiological basis of improved fruit retention, we monitored hormonal dynamics in nascent fruits during the critical first week post-pollination. In the cross-pollinated group, the hormonal profile underwent a distinct and favorable reprogramming. Levels of the key growth-promoting hormones, auxin (IAA) and zeatin (ZT), exhibited a robust and synergistic upregulation. This accumulation followed a precise temporal pattern, initiating shortly after pollination and culminating in a peak concentration at 5 DPP (Fig. 5). This surge contrasts sharply with self-pollinated fruits, where these hormones remained at basal levels. Simultaneously, levels of Abscisic Acid (ABA), a promoter of senescence and abscission, declined to their lowest point at 5 DPP in the cross-pollinated group. This shift—characterized by elevated IAA/ZT and minimized ABA—creates an optimal physiological environment for rapid cell division and effectively suppresses the activation of the abscission zone during the critical window of fruit set establishment.

Proteomic Reprogramming Activates Reproductive and Metabolic Pathways

Quantitative proteomic analysis of ovaries at 7 DPP provided a molecular snapshot of the fertilization response. We identified 367 differentially expressed proteins (DEPs) between cross-pollinated and self-pollinated groups (249 upregulated, 118 downregulated) (Fig. 6), indicating a massive transcriptional and translational shift. Gene Ontology (GO) enrichment analysis revealed a significant activation of specific reproductive processes in the cross-pollinated group. Notable enriched terms included “pollen recognition” (GO:0048544), “pollen-pistil interaction” (GO:0009875), and “pollen tube guidance”, evidenced by the specific upregulation of guidance-related proteins such as V4S0L0.

Further KEGG pathway analysis highlighted a global metabolic reconfiguration. The “Phenylpropanoid biosynthesis” pathway was the most significantly enriched ($p = 3.13\times10^{-6}$), with 12 out of 15 associated proteins (including Phenylalanine Ammonia-Lyase [PAL], 4-Coumarate: CoA Ligase [4CL], and Cinnamyl Alcohol Dehydrogenase [CAD]) showing upregulation. Additionally, pathways related to “Plant hormone signal transduction” and “Brassinosteroid biosynthesis” were significantly enriched, corroborating the physiological hormone data. These molecular alterations suggest a multi-layered mechanism wherein cross-pollination not only triggers fertilization but also activates metabolic pathways essential for structural strengthening and growth signaling.

Discussion

Self-incompatibility (SI) is a major biological constraint that limits the yield potential of pummelo [39–42], particularly in *C. maxima* ‘Tomentosa’, rendering it reliant on external pollen sources for commercial viability. Our results demonstrate that the selection of an appropriate pollen donor is not merely a matter of convenience but a critical factor for surmounting this barrier. Among the tested cultivars, ‘Jinju’ Pomelo proved to be an elite pollinator, significantly outperforming the industry-standard ‘Hongrou’ pomelo. This superior performance appears to originate from a combination of high pollen vigor—characterized by rapid germination and tube growth—and high genetic compatibility that facilitates successful fertilization. The rapid germination observed in ‘Jinju’ Pomelo pollen allows it to outcompete biological degradation processes in the style, ensuring fertilization occurs while the ovule is still viable.

Physiologically, the transition from flower to fruit is governed by complex hormonal crosstalk. Our data reveal that compatible cross-pollination triggers a specific “hormonal pulse” characterized by the accumulation of auxin and cytokinin (ZT) at 5–7 DPP, coinciding with the suppression of ABA. This temporal synchronization is vital. High levels of IAA and ZT are known to drive the rapid cell division required for initial fruit enlargement [43–45], establishing the sink strength of the young fruit. This strong sink activity draws nutrients to the developing fruit, preventing starvation-induced abscission. Concurrently, the downregulation of ABA prevents the transcriptional activation of hydrolytic enzymes like cellulases and polygalacturonases in the pedicel abscission zone [46]. The failure of self-pollinated fruits to mount this coordinated hormonal response likely results in the activation of the abscission zone

and subsequent fruit drop. The identification of this 5–7 day window as a critical period for hormonal regulation offers a practical target for exogenous hormone applications in orchard management.

At the molecular level, our proteomic data provides novel mechanistic insights into how cross-pollination reinforces fruit set. The specific upregulation of pollen tube guidance proteins in the cross-pollinated group correlates perfectly with the extensive tube growth observed microscopically, confirming that the maternal tissue actively facilitates the growth of compatible pollen via chemotropic signals. Perhaps most intriguingly, the activation of the phenylpropanoid pathway suggests a “structural-chemical” dual mechanism for fruit retention. Structurally, the upregulation of lignin biosynthesis enzymes (PAL, 4CL, CAD) likely leads to increased lignification of the fruit pedicel vascular tissue. This enhanced lignification provides mechanical resistance to physical abscission and ensures robust vascular connections for nutrient transport [7, 47]. Chemically, this pathway competes for *trans*-cinnamic acid, a precursor shared with the biosynthesis of Jasmonic Acid (JA). By diverting metabolic flux toward henylpropanoids/lignin, the plant may effectively reduce the synthesis of JA, a hormone often associated with senescence, stress responses, and abscission signaling. This “double-lock” mechanism-enhancing mechanical strength while attenuating abscission signals-likely underpins the exceptionally high branch fruit retention rates observed with ‘Jinju’ Pomelo pollination.

Conclusions

In summary, this study identifies ‘Jinju’ Pomelo as an elite pollinator for *C. maxima* ‘Tomentosa’, capable of significantly enhancing both fruit set and developmental quality. The underlying mechanism is multifaceted, involving: (1) superior pollen viability and compatibility that ensures rapid fertilization; (2) a synergistic hormonal reprogramming (elevated IAA/ZT, reduced ABA) that drives growth and suppresses abscission during the critical establishment window; and (3) a molecular response characterized by the activation of reproductive guidance proteins and the phenylpropanoid pathway, which strengthens fruit retention. These findings provide a solid scientific foundation for the widespread adoption of ‘Jinju’ Pomelo as a pollinator in commercial orchards, offering a sustainable strategy to boost yields in the ‘Juhong’ industry.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Funding

This research was funded by the Special Project of Guangxi Special Crops Experiment Station (Gui TS2022013), Gui Zhongnan Comprehensive Experimental Station of the National Modern Agricultural Industry Technology System (Citrus) (CARS-26), and Breeding and Utilization of New Processing-type Citrus Varieties (Gui Ke AB21196044).

Author Contribution

Y.L.: Investigation; Methodology; Formal analysis; Writing-original draft. H.Q.: Methodology; Formal analysis; Data curation; Writing-original draft.W.X.:Formal Analysis; Visualization; Writing-Review and Editing.X.Z.:Data curation; Formal Analysis. Z.O.:Validation; Formal Analysis. X.S.: Methodology; Data curation. J.L.: Investigation; Formal analysis. J.L.: Investigation; Methodology. X.C.: Data curation; Formal analysis. C.C.: Conceptualization; Project administration; Data curation; Funding acquisition; Supervision; Writing-review and editing.All authors have read and agreed to the published version of the manuscript.

Acknowledgement

We are grateful to Professor Changqing Chang for his valuable suggestions and assistance with the manuscript revision.

Data Availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

References

1. Duan L, Guo L, Dou L-L, Yu K-Y, Liu E-H, Li P. Comparison of chemical profiling and antioxidant activities of fruits, leaves, branches and flowers of ‘Tomentosa’. J Agric Food Chem. 2014;62(44):10754–63. https://doi.org/10.1021/jf5036355.
2. Li P-B, Chen P, Wu H, Wang Y-G, Rao H-Y, Su W-W. Discussion and prediction of application prospects of Citri Grandis Exocarpium on COVID-19 based on literature analysis and molecular docking. Chin Tradit Herb Drugs. 2020;51(9):2368–78. https://doi.org/10.7501/j.issn.0253-2670.2020.09.011.
3. Honsho C. Self-incompatibility related to seedless fruit production in citrus plants. Hortic J. 2023;92(1):1–12. https://doi.org/10.2503/hortj.QH-001.
4. Montalt R, Vives MC, Navarro L, Ollitrault P, Aleza P. Parthenocarpy and self-incompatibility in mandarins. Agronomy. 2021;11(10):2023. https://doi.org/10.3390/agronomy11102023.

5. Liang M, Cao Z, Zhu A, Liu Y, Tao M, Yang H, Xu Q, Wang S, Liu J, Li Y, Chen C, Xie Z, Deng C, Ye J, Guo W, Xu Q, Xia R, Larkin RM, Deng X, Bosch M, Franklin-Tong VE, Chai L. Evolution of self-compatibility by a mutant Sm-RNase in citrus. Nat Plants. 2020;6(2):131–42. https://doi.org/10.1038/s41477-020-0597-3.
6. Alinezhad Jahromi H, Zarei A, Mohammadkhani A. Analysis the effects of pollen grain sources on the fruits set and their characteristics of 'Clementine' mandarin using microscopic and molecular approaches. Sci Hortic. 2019;249:347–54. https://doi.org/10.1016/j.scienta.2019.02.010.
7. Wang S, Long C, Liu H, Pan L, Yang S, Zhao J, Jiang Y, Bei X. Comparative physiochemical and transcriptomic analysis reveals the influences of cross-pollination on ovary and fruit development in pummelo (Citrus maxima). Sci Rep. 2023;13(1):19081. https://doi.org/10.1038/s41598-023-46058-3.
8. Aleza P, Juarez J, Cuenca J, Ollitrault P, Navarro L. Recovery of citrus triploid hybrids by embryo rescue and flow cytometry from 2x × 2x sexual hybrid application to extensive breeding programs. Plant Cell Rep. 2010;29(9):1023–34. https://doi.org/10.1007/s00299-010-0888-7.
9. Montalt R, Prosper L, Vives MC, Navarro L, Ollitrault P, Aleza P. Breakdown self-incompatibility in citrus by temperature stress, bud pollination and polyploidization. Agriculture. 2022;12(2):273. https://doi.org/10.3390/agriculture12020273.
10. Atmowidi T, Cholis MN, Maulana A, Priawandiputra W, Kahono S. Effectiveness of pollinator insects in increasing fruit formation of pummelo (Citrus maxima(Burm.) Merr). Aust J Crop Sci. 2022;16(9):1078–83. https://search.informit.org/doi/10.3316/informit.846583381086973.
11. Ezura K, Nomura Y, Ariizumi T. Molecular, hormonal, and metabolic mechanisms of fruit set, the ovary-to-fruit transition, in horticultural crops. J Exp Bot. 2023;74(12):3639–54. https://doi.org/10.1093/jxb/erad214.
12. Fenn MA, Giovannoni JJ. Phytohormones in fruit development and maturation. Plant J. 2021;105(2):307–20. https://doi.org/10.1111/tpj.15112.
13. Ruan Y-L, Patrick JW, Bouzayen M, et al. Molecular regulation of seed and fruit set. Trends Plant Sci. 2012;17(11):656–65. https://doi.org/10.1016/j.tplants.2012.06.005.
14. He H, Yamamuro C. Interplays between auxin and GA signaling coordinate early fruit development. Hortic Res. 2022;9:uhab078. https://doi.org/10.1093/hr/uhab078.
15. Bermejo A, Granero B, Mesejo C, et al. Auxin and gibberellin interact in citrus fruit set. J Plant Growth Regul. 2017;36(4):1024–33. https://doi.org/10.1007/s00344-017-9748-9.
16. Sharif R, Su L, Chen X-H, et al. Hormonal interactions underlying parthenocarpic fruit formation in horticultural crops. Hortic Res. 2022;9:uhab024. https://doi.org/10.1093/hr/uhab024.
17. Zhang H-Y, Zhao J-X, Zhang J-L, et al. Low-concentration exogenous 3-indolecacetic acid improves fruit-setting rate of Marsdenia tenacissimaby inhibiting the expression of embryo abortion-related genes. Gene. 2024;893:147930. https://doi.org/10.1016/j.gene.2023.147930.
18. Kim JS, Ezura K, Lee J, et al. The inhibition of SlIAA9 mimics an increase in endogenous auxin and mediates changes in auxin and gibberellin signalling during parthenocarpic fruit development in

tomato. J Plant Physiol. 2020;252:153238. https://doi.org/10.1016/j.jplph.2020.153238.

19. Tian Y, Xin W, Lin J, et al. Auxin coordinates achene and receptacle development during fruit initiation in Fragaria vesca. Front Plant Sci. 2022;13:929831. https://doi.org/10.3389/fpls.2022.929831.
20. Xie R, Ge T, Zhang J, et al. The molecular events of IAA inhibiting citrus fruitlet abscission revealed by digital gene expression profiling. Plant Physiol Biochem. 2018;130:192–204. https://doi.org/10.1016/j.plaphy.2018.07.006.
21. Niharika N, Singh NB, Singh A, et al. Mitigating strategies of gibberellins in various environmental cues and their crosstalk with other hormonal pathways in plants: a review. Plant Mol Biol Rep. 2021;39(4):34–49. https://doi.org/10.1007/s11105-020-01231-0.
22. Fan D, Yang L, Jin J, et al. Hormonal and transcriptomic analysis reveal gibberellin-induced fruit set in jujube(Ziziphus jujuba Mill). Plant Mol Biol Rep. 2022;40:163–75. https://doi.org/10.1007/s11105-021-01308-4.
23. Chen C, Chen H, Chen Y, et al. Joint metabolome and transcriptome analysis of the effects of exogenous GA3 on endogenous hormones in sweet cherry and mining of potential regulatory genes. Front Plant Sci. 2022;13:1041068. https://doi.org/10.3389/fpls.2022.1041068.
24. Shinozaki Y, Beauvoit BP, Takahara M, et al. Fruit setting rewires central metabolism via gibberellin cascades. Proc Natl Acad Sci U S A. 2020;117(38):23970–81. https://doi.org/10.1073/pnas.2011859117.
25. Shinozaki Y, Ezura H, Ariizumi T. The role of ethylene in the regulation of ovary senescence and fruit set in tomato (Solanum lycopersicum). Plant Signal Behav. 2018;13(4):e1146844. .https://doi.org/10.1080/15592324.2016.1146844.
26. Yu T, Yang Y, Wang H, et al. The variations of C/N/P stoichiometry, endogenous hormones, and non-structural carbohydrate contents in Michelia maudiae'Rubicunda' flower at five development stages. Horticulturae. 2023;9(11):1198. https://doi.org/10.3390/horticulturae9111198.
27. An J, Althiab Almasaud R, Bouzayen M, et al. Auxin and ethylene regulation of fruit set. Plant Sci. 2020;292:110381. https://doi.org/10.1016/j.plantsci.2019.110381.
28. Qiu Z, Wen Z, Hou Q, et al. Cross-talk between transcriptome, phytohormone and HD-ZIP gene family analysis illuminates the molecular mechanism underlying fruitlet abscission in sweet cherry (Prunus aviumL). BMC Plant Biol. 2021;21(1):173. https://doi.org/10.1186/s12870-021-02940-8.
29. Shi Y, Song B, Liang Q, et al. Molecular regulatory events of flower and fruit abscission in horticultural plants. Hortic Plant J. 2023;9(5):867–83. https://doi.org/10.1016/j.hpj.2023.03.008.
30. Zhou H, Wang X, Amar MH, et al. Abscisic acid induces PpeKIL1 to terminate fruit growth and promote fruit abortion in peach (Prunus persica). Plant Physiol Biochem. 2024;212:108761. https://doi.org/10.1016/j.plaphy.2024.108761.
31. Hu J, Israeli A, Ori N, Sun TP. The interaction between DELLA and ARF/IAA mediates crosstalk between gibberellin and auxin signaling to control fruit initiation in tomato. Plant Cell. 2018;30(8):1710–28. https://doi.org/10.1105/tpc.18.00363.

32. Su L, Chen J, Wu Y, et al. Cytokinin, gibberellin and auxin regulate parthenocarpy in cucumber via modulating downstream auxin signaling network. Plant Physiol Biochem. 2025;229. https://doi.org/10.1016/j.plaphy.2025.110492. Pt B):110492.
33. Guo K-B, Qiao G, Qiu Z-L, et al. The fruit dropping characters of sweet cherry and its interior causes in insufficient chilling zone. Russ J Plant Physiol. 2020;67(1):94–102. https://doi.org/10.1134/S1021443720010070.
34. Hu Z, Lan S, Zhao N, et al. Soft-X-irradiated pollens induce parthenocarpy in watermelon via rapid changes of hormone-signalings and hormonal regulation. Horticulturae. 2019;250:217–28. https://doi.org/10.1016/j.scienta.2019.02.036.
35. Ogawa M, Takisawa R. Effect of exogenous plant hormones on parthenocarpy and fruit quality in tropical squash (Cucurbita moschataL). Hortic J. 2022;91(4):508–13. https://doi.org/10.2503/hortj.UTD-368.
36. Chae K, Lord EM. Pollen tube growth and guidance: roles of small, secreted proteins. Ann Bot. 2011;108(4):627–36. https://doi.org/10.1093/aob/.
37. Mizuta Y, Higashiyama T. Chemical signaling for pollen tube guidance at a glance. J Cell Sci. 2018;131(10):jcs208447. https://doi.org/10.1242/jcs.208447.
38. Liu X-F, Sun M-X, Peng X-B. Molecular basis of pollen tube guidance and avoidance of polytubey fertilization. Plant Sci J. 2023;41(1):128–37. https://doi.org/10.11913/PSJ.2095-0837.22082.
39. Zhang S, Liang M, Wang N, Xu Q, Deng X, Chai L. Reproduction in woody perennial citrus: an update on nuclear embryony and self-incompatibility. Plant Reprod. 2018;31(1):43–57. https://doi.org/10.1007/s00497-018-0327-4.
40. Kim JH, Handayani E, Wakana A, et al. Distribution and evolution of Citrus accessions with S3 and/or S11 alleles for self-incompatibility with an emphasis on sweet orange [Citrus sinensis (L.) Osbeck; SfS3 or SfS3sm]. Genet Resour Crop Evol. 2020;67(8):2101–17. https://doi.org/10.1007/s10722-020-00964-x.
41. Liang M, Yang W, Su S, et al. Genome-wide identification and functional analysis of S-RNase involved in the self-incompatibility of citrus. Mol Genet Genomics. 2017;292(2):325–41. https://doi.org/10.1007/s00438-016-1279-8.
42. Yamamoto M, Kubo T, Tominaga S. Self- and cross-incompatibility of various citrus accessions. J Jpn Soc Hortic Sci. 2006;75(5):372–8. https://doi.org/10.2503/jjshs.75.372.
43. Zhang SY. Progress in the early auxin response gene Aux/IAA. Mol Plant Breed. 2013;11(29):1211–8. https://doi.org/10.5376/mpb.cn.2013.11.0029.
44. Cao M, Chen R, Li P, et al. TMK1-mediated auxin signalling regulates differential growth of the apical hook. Nature. 2019;568(7751):1–4. https://doi.org/10.1038/s41586-019-1069-7.
45. Luo J, Zhou JJ, Zhang JZ. Aux/IAA Gene Family in Plants: Molecular Structure, Regulation, and Function. Int J Mol Sci. 2018;19(1):259. https://doi.org/10.3390/ijms19010259.
46. Ying P-Y. Study on the identification and molecular characterization of LcIDL1 and transcription factor LcKNOX23 involved in fruit abscission in litchi. South China Agricultural University,

Guangzhou, China; 2017.

47. Gupta H, Goyal M, Kapoor R, Mittal A. Role of lignin metabolism and associated metabolites for lodging resistance in oat (Avena sativaL). Cereal Res Commun. 2025;53(1):261–73. https://doi.org/10.1007/s42976-024-00537-z.

Figures

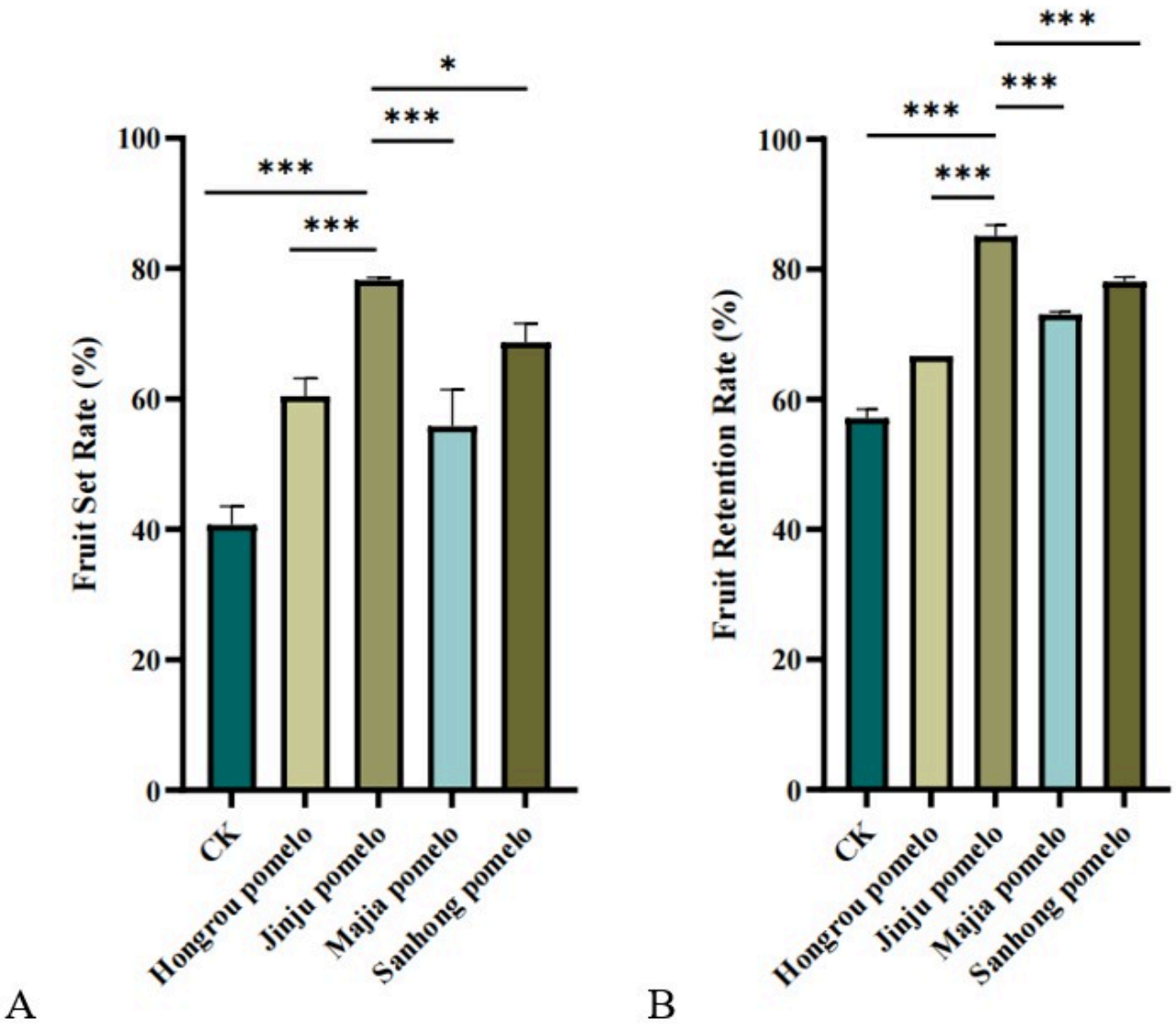

Figure 1

Fruit set and branch retention rates following cross-pollination. (A) Initial fruit set rate (%) of *C. maxima* 'Tomentosa' after self- and cross-pollination with different pomelo cultivars. (B) Effective fruiting branch rate (%) at harvest. Data are presented as mean ± SD. Asterisks indicate significant differences ($p < 0.05$, Duncan's test).

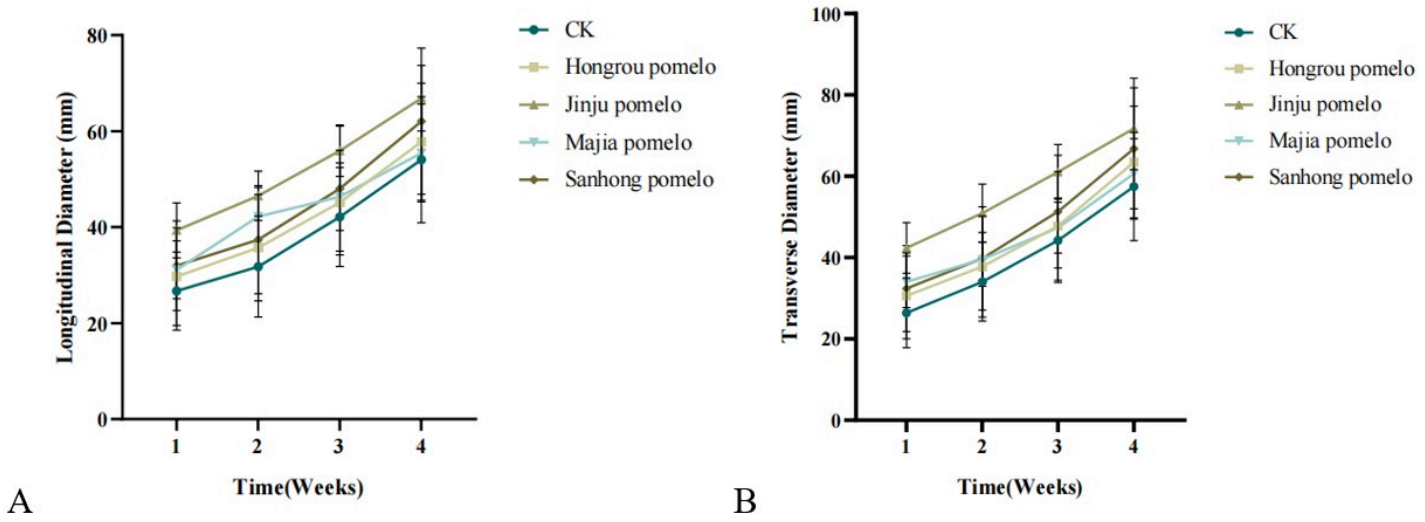

Figure 2

Fruit morphometrics of *C. maxima* 'Tomentosa' following cross-pollination. (A)Vertical and (B) transverse diameters of developing fruits from self- and cross-pollination treatments. Data are shown as mean ± SD.

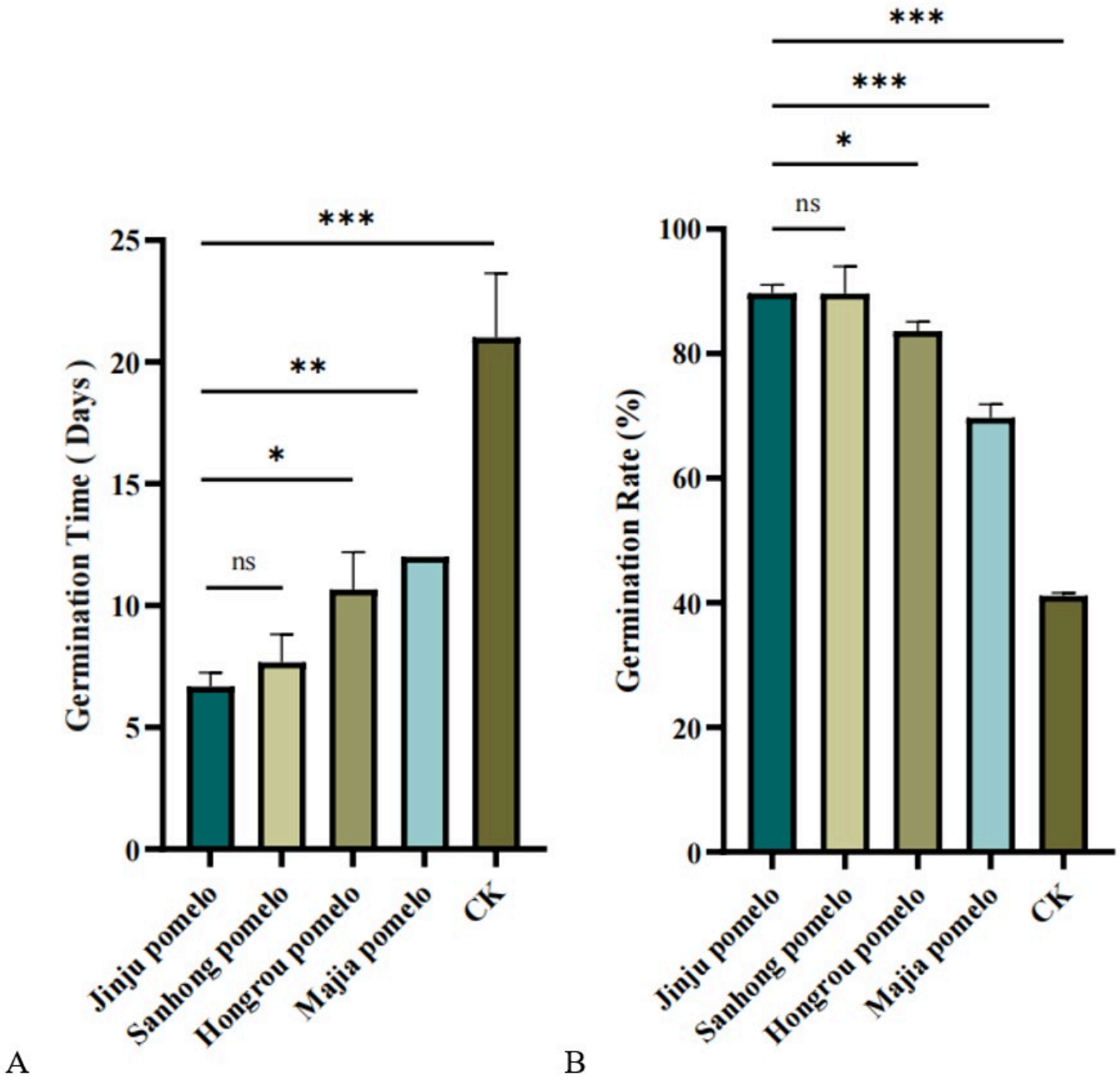

Figure 3

Pollen germination characteristics of different pomelo cultivars. (A) Germination latency (time to first germination) and (B) final germination rate (%) of pollen from four donor cultivars and self-pollen of *C. maxima* 'Tomentosa'. Values are mean ± SD. Asterisks indicate significant differences ($p < 0.05$).

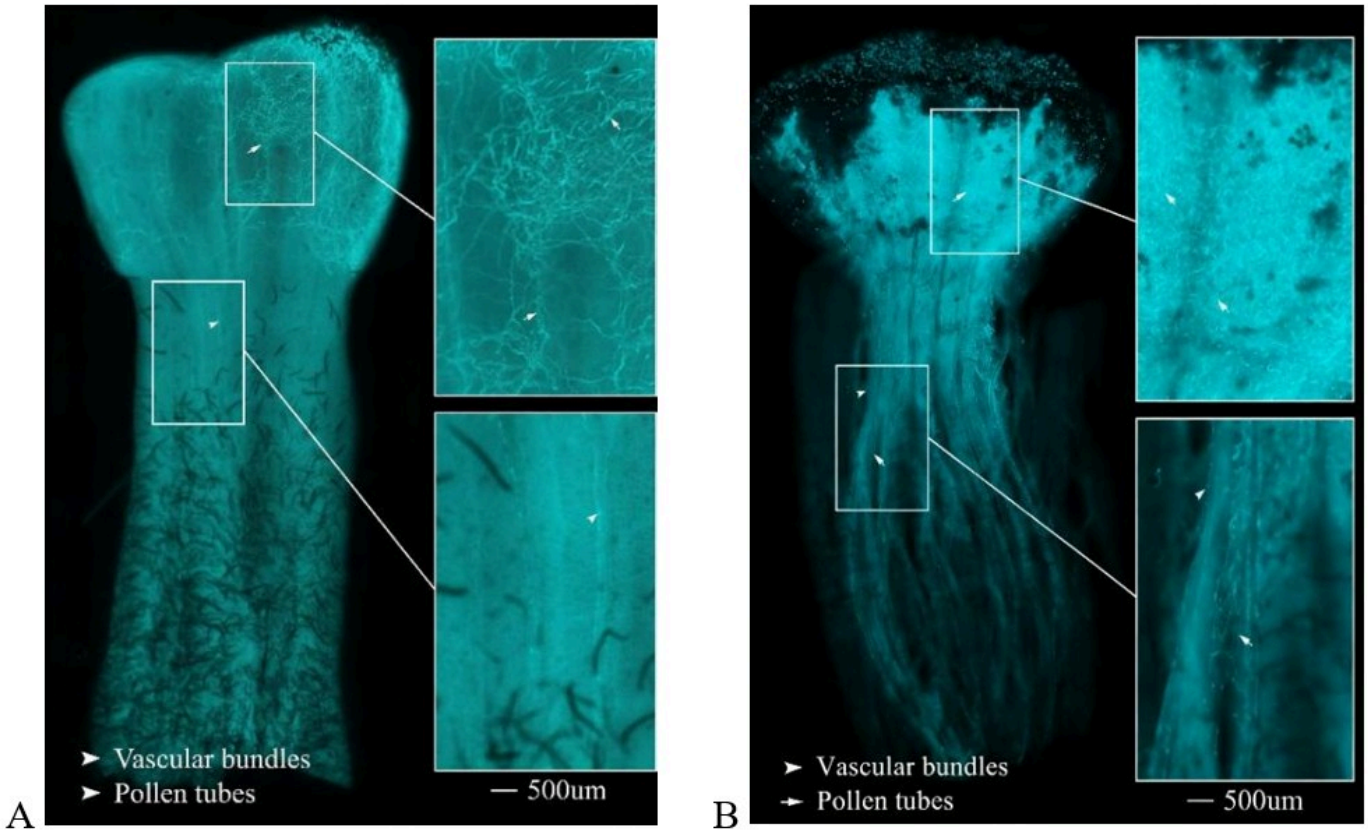

Figure 4

Fluorescence micrographs of pollen tube growth in styles at 7 days post-pollination. (A) Self-pollinated style showing arrested pollen tubes in the upper style. (B) Style pollinated with ‘Jinju’ Pomelo pollen, showing extensive pollen tube growth reaching the ovary base. Pollen tubes are stained with aniline blue and visualized under UV light. Scale bars = 500 μm.

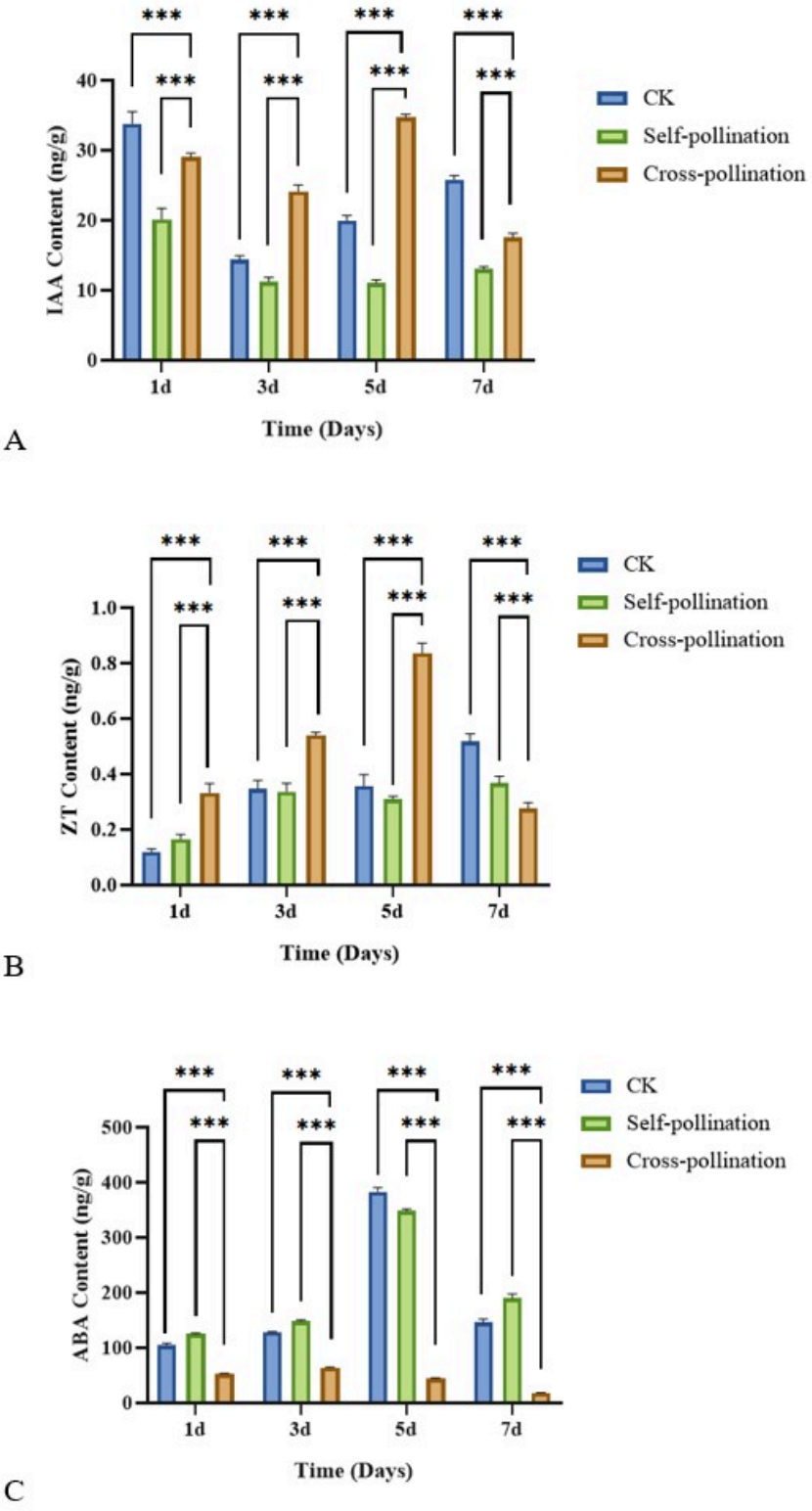

Figure 5

Dynamics of endogenous hormones in young fruits after pollination. Concentrations of (A) auxin (IAA), (B) zeatin (ZT), and (C) abscisic acid (ABA) in ovaries from self-pollinated and 'Jinju' Pomelo-pollinated flowers at 1, 3, 5, and 7 days post-pollination. Data are mean ± SD (n = 3). Asterisks indicate significant differences between treatments at each time point (* $p < 0.05$).

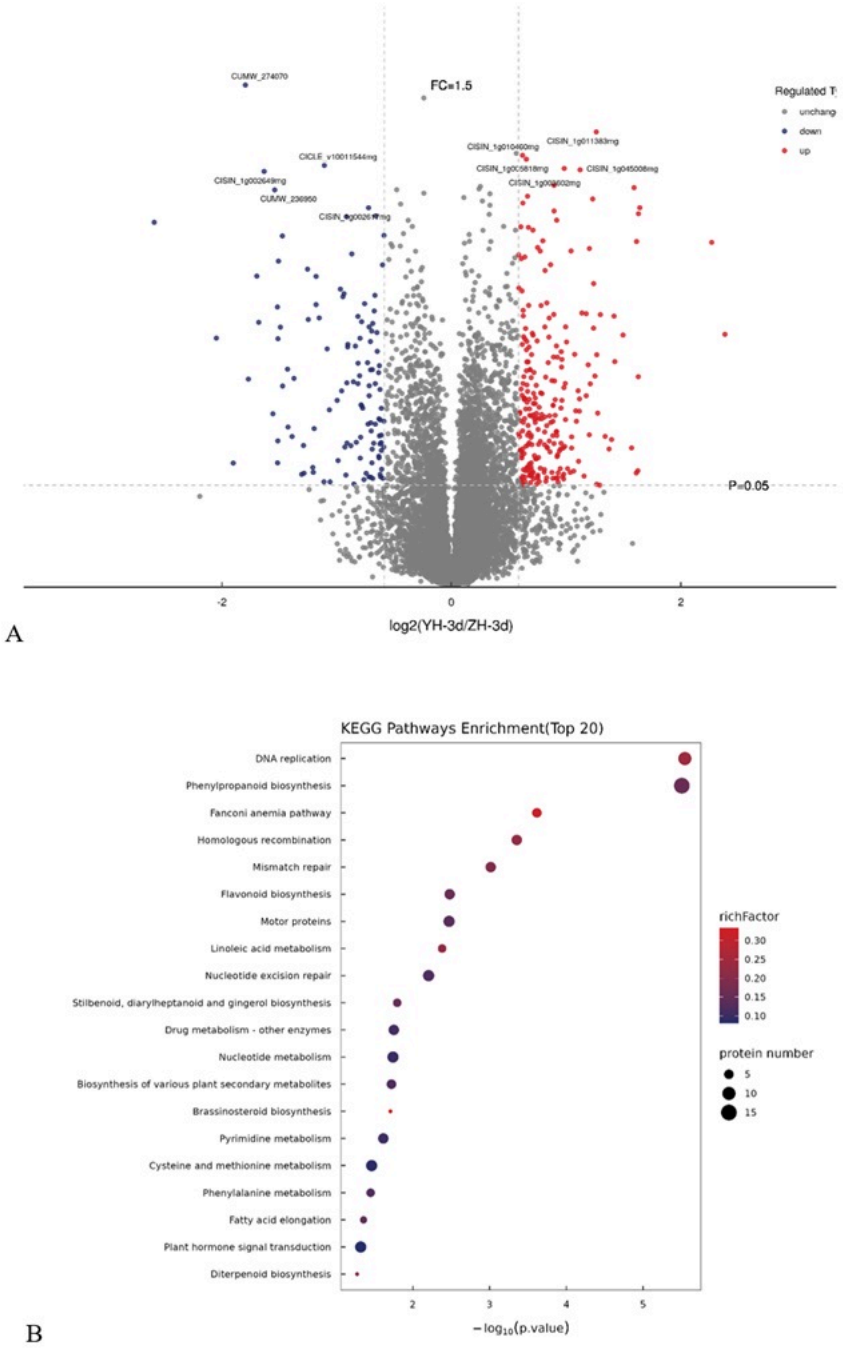

Figure 6

Quantitative proteomic analysis of ovaries at 7-day post pollination. (A) Volcano plot of differentially expressed proteins (DEPs) between cross-pollinated and self-pollinated groups. (B) KEGG pathway enrichment analysis of DEPs.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Fig.S1.pdf
- Fig.S2.pdf