

Expression profiling of Auxin and GA signaling genes during fruit set in *Zanthoxylum armatum*

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Abstract

Zanthoxylum armatum DC. is an important economic crop in Southwest China, but severe fruit drop and low fruit set rate reduce yields. Identification of key genes regulating fruit set in *Z. armatum* could provide new insights for addressing fruit abscission. In this study, we examined the morphological and anatomical characteristics of pistils and young fruits in two *Z. armatum* cultivars, 'Jiuyeqing' and 'Prickleless'. Both cultivars exhibited unisexual flowers with apomictic traits and similar fruit morphology as well as anatomy. Transcriptome data were utilized to identify auxin and gibberellin biosynthetic and signaling genes. A total of 21 *Aux/IAAs*, 19 *ARFs*, 2 *GA20oxs*, 3 *GA2OXs*, *GID1*, and 6 *DELLA* genes were differentially expressed in male and female flowers, suggesting their candidate roles in regulating fruit set. Phylogenetic analysis revealed that *Aux/IAA* family members clustered into two clades with seven subfamilies, while *ARFs* were divided into four subclasses. *ZaGA20oxs*, *ZaGA2oxs*, *ZaGID1*, and *ZaDELLAs* showed high homology to their orthologs in tomato and cucumber. Quantitative real-time PCR (qRT-PCR) analysis showed that auxin-related *ZaLAA8*, *ZaLAA11*, *ZaLAA20*, *ZaARF1*, *ZaARF5*, and *ZaARF10* exhibited similar expression trends during fruit set in both cultivars, whereas other genes (e.g., *ZaLAA6*, *ZaLAA7*, *ZaARF9*, *ZaARF18*) displayed divergent expression. Gibberellin-related *ZaGA20ox2*, *ZaGA2OX2*, *ZaGA2OX4*, *ZaDELLA1*, and *ZaDELLA3* were significantly differentially expressed during pistil development and fruit initiation across cultivars. This study provides insights into the molecular mechanisms of phytohormone-mediated fruit development and offers a theoretical foundation for breeding high-yield varieties.

Keywords: auxin; expression profiling; fruit set; gibberellin; *Zanthoxylum armatum*

Introduction

Zanthoxylum is a shrub or small tree of the Rutaceae family, which is native to China. There are approximately 250 species distributed in tropical and subtropical regions of Asia, America, Africa and Oceania,

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with 45 species and 13 varieties in China (Sun *et al.*, 1996). The two primary cultivated species are *Z. bungeanum* Maxim. (commonly known as “red prickly ash” due to its red pericarp at maturity) and *Z. armatum* DC. (referred to as “green prickly ash” for its green pericarp). *Z. armatum* is cold-sensitive and predominantly cultivated in southwestern China. Currently, the planting area exceeds 4 million hectares (\approx 600 million mu), with an annual total output value of 30 billion yuan, making it a crucial economic crop supporting the rural revitalization strategy in this region. Notably, two superior *Z. armatum* cultivars, ‘Jiuyeqing’ and ‘Prickleless’ selected from bud mutation, have become the dominant varieties in Chongqing, favored for their high yield, rich aroma, authentic numbing flavor, and high oil content, demonstrating significant market potential (Deng *et al.*, 2008; Yang *et al.*, 2019; Zhang *et al.*, 2020). However, production faces challenges, including severe flower and fruit drop coupled with low natural fruit-setting rates, which requires extensive use of plant growth regulators, significantly increasing labor and production costs while reducing economic benefits. Therefore, developing effective strategies to improve fruit-setting rate is imperative, and elucidating the molecular mechanisms underlying fruit-setting and development in *Z. armatum* will provide an essential theoretical foundation to address this critical industrial problem.

Fruit set, the complete developmental process from flowering to fruit initiation, represents a critical transition from vegetative to reproductive growth in plants, involving complex signal transduction pathways, gene regulatory networks, and extensive interactions between biotic and abiotic factors (Karlova *et al.*, 2014). Previous studies demonstrate that flowering and fruit setting are predominantly regulated by endogenous hormones, particularly auxin and gibberellins which play central roles during this process (Ruan *et al.*, 2012; Sotelo-Silveira *et al.*, 2014). As the first identified phytohormone, auxin exerts crucial regulatory effects on cell expansion, organogenesis, fruit development and maturation, as well as stress responses (Kazan *et al.*, 2009). Serrani *et al.* (2008) demonstrated that exogenous auxin application could induce parthenocarpic fruit set and promote fruit enlargement in unpollinated tomato ovaries. Cytological observations revealed an increase in the number of pericarp cell layers, indicating that auxin promoted cell division. Auxin-induced fruit set and cell expansion have also been observed in pepper (Heuvelink *et al.*, 2001). In the auxin signaling pathway, Aux/IAA proteins serve as key regulators. *SLAA9* mutation can lead to anther defects and parthenocarpy in tomato (Mazzucato *et al.*, 2015). Auxin response factors (*ARFs*), functioning as transcription factors, modulate auxin-responsive gene expression by specifically binding to auxin response elements (TGTCTC) in the promoter region, thereby governing fruit set, development and maturation (Li *et al.*, 2024). ARF family in *Arabidopsis* comprises five subfamilies (I-V), with most members acting as transcriptional repressors, while AtARF5-8 and AtARF19 function as activators (Liu *et al.*, 2011). In eggplant, silencing of *SmARF8* silencing induces parthenocarpy (Du *et al.*, 2016). In tomato, SlARF5 promotes cell division but inhibits expansion during fruit development (Liu *et al.*, 2018). Notably, the regulatory mechanisms of auxin signaling components in *Z. armatum* fruit development remain uncovered, representing a significant research gap for this economically important crop.

Gibberellins (GAs), a class of plant hormones predominantly localized in actively growing tissues, play pivotal regulatory roles in fruit growth, development, and maturation (Fenn *et al.*, 2021). In most fruit trees, GAs biosynthesis post-ovule fertilization serves as crucial signals for ovary development, and endogenous GA signaling initiate fruit formation. Previous studies demonstrate that exogenous GA application significantly enhances the length, size, diameter, and volume of fruit cells, indicating its involvement in fruit morphogenesis through cell expansion (Li *et al.*, 2024). De Jong *et al.* (2009) revealed that GA could induce parthenocarpic fruit formation in tomato. GA biosynthesis involves three key enzymes: gibberellin 20-oxidases (GA20oxs), gibberellin 3-oxidases (GA3oxs), and gibberellin 2-oxidases (GA2oxs). The increase in GA content in the ovary after pollination is due to the elevated transcription of GA20ox, which enhances the activity of GA20-oxidase. Increased GA20ox and GA3ox activities promote parthenocarpy, while GA2oxs inhibit seedless fruit formation. Notably, *GA20ox* gene deletion leads to extreme dwarfism and complete sterility in *Arabidopsis*

(Plackett *et al.*, 2012). DELLA proteins function as negative regulators of GA signaling, suppressing ovary growth before pollination and fertilization. GA binds to the GID1 receptor to form a complex that degrades DELLA proteins, thereby regulating DELLA-dependent plant growth and development. *DELLA* mutants in *Arabidopsis* and tomato exhibit parthenocarpic fruit formation (Guan *et al.*, 2024). However, compared with model species, knowledge on GA signaling components regulating fruit set in *Z. armatum* remains remarkably limited (Zhang *et al.*, 2020).

In this study, we investigated fruit morphological and anatomical features across developmental stages of two major *Zanthoxylum* cultivars in southwest China, that are 'Jiuyeqing' and 'Prickleless'. RNA-Seq analysis identified candidate genes related to auxin and gibberellin that regulate fruit set. The phylogenetic relationships were analyzed using bioinformatics tools, and their expression patterns during pistil development and fruit formation were determined by qRT-PCR. The findings provide insights into the molecular mechanisms of auxin and gibberellin in fruit setting and development, and also lay a theoretical foundation for breeding high-yield *Zanthoxylum* varieties.

Materials and Methods

Biological material

Two *Zanthoxylum* cultivars, 'Jiuyeqing' (JQY) and 'Prickleless' (PL) were used in this experiment, which were cultivated in the Germplasm Resource Nursery of Chongqing University of Arts and Sciences. Fruit sampling was conducted according to cultivar-specific phenological stages. For the JQY cultivar, fruits were collected at three developmental stages, including ovule primordium formation period (S1) on March 15, ovule formation period (S2) on March 29, and ovule expansion stage (S3) on April 14. Meanwhile, for the PL cultivar, fruits were harvested at the corresponding stages, S1 on March 5, S2 on March 19, and S3 on April 4. After collection, samples were divided into two groups. One part was preserved in fixative solution for histological sectioning analysis, and another was flash-frozen in liquid nitrogen and then transferred to -80 °C ultra-low temperature storage for molecular analysis.

Morphological observation

The collected samples were fixed in 4% paraformaldehyde (PFA) for 24-72 hours, followed by gradient dehydration in 15%, 20%, and 30% (w/v) sucrose solutions until complete sedimentation. The tissues were embedded in OCT embedding medium on glass slides and sectioned into 10µm-thick slices using a BL-800A cryostat. The sections were stained with 0.1% (w/v) safranin solution for 1 hour, then successively dehydrated in 50%, 70%, and 80% (v/v) ethanol (1 minute each), counterstained with 1% (w/v) fast green solution for 1 minute, and finally mounted with neutral balsam. Histological observations and imaging were performed under a LEICA DM 3000 microscope.

Screening of fruit set related genes in Z. armatum

Candidate genes were screened from the 'Jiuyeqing' floral transcriptome database (Zhang *et al.*, 2020) using "IAA", "ARF", "GID", "DELLA", "GA20ox", and "GA2OX" as key search terms. The open reading frames (ORFs) were identified using ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi>), followed by sequence validation through ExPASy (<https://web.expasy.org/translate/>) for translation analysis and NCBI BLAST (<https://www.ncbi.nlm.nih.gov/>) for homology searches.

Phylogenetic analysis

Protein sequences of auxin- and GA-related genes involved in biosynthesis and signal transduction were retrieved from NCBI and the Arabidopsis Information Resource (TAIR, <http://www.arabidopsis.org/>). Phylogenetic analysis was performed by using MEGA 6.0 software with the neighbor-joining method to construct evolutionary relationships among these sequences.

Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from samples using the OminiPlant RNA Kit (CW BIO, Beijing, China). RNA integrity was verified by 1% agarose gel electrophoresis and concentration was measured by using NanoDrop 1000 (Thermo, USA). After DNase I treatment, cDNA was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA). Gene-specific primers for qRT-PCR were designed using online primer design software (<http://www.genscript.com.cn/technologysupport/onlinetools>), and listed in Table 1. Quantitative PCR was performed using UltraSYBR Mixture (CW BIO, Beijing, China) on a qTower 2.2 real-time PCR system (Analytic Jena, Leipzig, Germany). ZaUBC was selected as the reference gene for relative quantification using the $2^{-\Delta\Delta C_t}$ method. All experiments were conducted in three technical replicates and three biological replicates for statistical reliability.

Table 1. Primer sequences used in qRT-PCR

Gene ID	Primer name	Primers Sequence(5'-3')
DN41039_c0_g2	<i>ZaIAA1F</i>	AGCTGAAGCTTGGACCACCAG
	<i>ZaIAA1R</i>	GCCGTTGTTGTTGTTGTTAATGGAAGA
DN41039_c0_g1	<i>ZaIAA2F</i>	TGGCCTCCAGTTCGTTCAATTTAGG
	<i>ZaIAA2R</i>	CTGCTGGGCTCATGAGTTGGT
DN39845_c0_g2	<i>ZaIAA3F</i>	ACCTCTCACAATGTTCCAGCAG
	<i>ZaIAA3R</i>	GGTGAACATCACCCAGGGTACATC
DN39845_c0_g1	<i>ZaIAA4F</i>	TGTTGCTCAGTCACCCAAGCC
	<i>ZaIAA4R</i>	AATTGGCGCCATCCCACA
DN37378_c0_g1	<i>ZaIAA5F</i>	GGACGGTGA CTGGATGCTTGT
	<i>ZaIAA5R</i>	TTTCTCCGCTGCCCTTGGAG
DN34185_c3_g3	<i>ZaIAA6F</i>	TGCGCATGCCTCCTATAAGACTTTG
	<i>ZaIAA6R</i>	CGTGAGCACAACTCAGATGATCCA
DN34185_c3_g1	<i>ZaIAA7F</i>	GGATCCTGCAGAGCCTTCTAAGC
	<i>ZaIAA7R</i>	CCAAGGTACATCTCCACAAGCA
DN33301_c0_g1	<i>ZaIAA8F</i>	TGTAGTCCTCGAGGGCCGTT
	<i>ZaIAA8R</i>	TCGCCGTCGACGAACATCTG
DN30847_c1_g2	<i>ZaIAA9F</i>	AGATCCACCTGTTGCTGGAAGC
	<i>ZaIAA9R</i>	TCTGCTCACTGCCATGGAACATC
DN30847_c1_g1	<i>ZaIAA10F</i>	AGATTGCGCGTGTGAAGAGC
	<i>ZaIAA10R</i>	GCATCATGAGTCGAGTCAAGCGA
DN30775_c0_g5	<i>ZaIAA11F</i>	TGGCCATGTACAATAATGGGT
	<i>ZaIAA11R</i>	TCAGAGCTGTCTGCATCCTT
DN30323_c0_g1	<i>ZaIAA12F</i>	TGTCCCGTGGCACATGTTTGT
	<i>ZaIAA12R</i>	TCTGCTTGCTGCTTCCAAAGGT
DN29744_c0_g4	<i>ZaIAA13F</i>	TAGTGCACCTGCTGCCAAGG
	<i>ZaIAA13R</i>	AGCACCAGGACCGGGTTTAC
DN29689_c1_g1	<i>ZaIAA14F</i>	AGCGAGCTGGATGGCATGAG
	<i>ZaIAA14R</i>	CACGATTCGGTGAACATCCTACAACA

DN28996_c2_g5	<i>ZaLAA15F</i>	CACAGTTGGTCAGTGTGGCTCT
	<i>ZaLAA15R</i>	GGGACATCACCGACTAGCATCC
DN28996_c2_g4	<i>ZaLAA16F</i>	GTGACTGGATGCTCGTGGGT
	<i>ZaLAA16R</i>	GCTCTTGCACTTCTCCATCGCT
DN28996_c2_g2	<i>ZaLAA18F</i>	TGCTTCTGCTTCTAGGGCTACTGA
	<i>ZaLAA18R</i>	TGGACGGCCATGATGTTCTTTCT
DN28021_c0_g1	<i>ZaLAA20F</i>	GTGCCTCCAACGCTGTCTCTT
	<i>ZaLAA20R</i>	TGAGATCCCAGTGCAGATTTGGG
DN27705_c2_g3	<i>ZaLAA21F</i>	TGGTGAAGTGGATGCTTGTGGT
	<i>ZaLAA21R</i>	CCATGGCTCTTGGAGCGAGT
DN27695_c2_g2	<i>ZaARF1F</i>	ACCGCGATGCATTTCGTTCT
	<i>ZaARF1R</i>	TGTCAGTGCCTGAATCCGTGAG
DN27843_c1_g3	<i>ZaARF2F</i>	TGGCGGTGAATTAATGTTCTCAAG
	<i>ZaARF2R</i>	ACTGCCATGGATCATCTCCAACA
DN34483_c1_g1	<i>ZaARF3F</i>	TGGGCCTCGACCGATGTTTG
	<i>ZaARF3R</i>	TGAAGTAAGCGGGTCAACTTGGG
DN34483_c1_g3	<i>ZaARF4F</i>	CGGAATCTACAGAGCAGTTGAGCA
	<i>ZaARF4R</i>	TGGCAGCTGCATTAGACCTTGG
DN37144_c0_g1	<i>ZaARF5F</i>	ACCCGCTACCATCAGAAAAGCC
	<i>ZaARF5R</i>	GCAGTCTGGATTGAAGATGAGTTGAGT
DN37973_c0_g1	<i>ZaARF6F</i>	TGGCCATCAGCAACAGAACA
	<i>ZaARF6R</i>	CCTCCAAGTGTAGCTCACCTG
DN37973_c0_g2	<i>ZaARF7F</i>	CTCAAGGTGCGGTGGGATGA
	<i>ZaARF7R</i>	GGGATCCATTGCAGGAGCCA
DN38175_c0_g5	<i>ZaARF8F</i>	GAAGGCACTCATCAGCATGTCTCT
	<i>ZaARF8R</i>	TCGGGAATTCTCCACCTTTGCC
DN39995_c1_g6	<i>ZaARF9F</i>	GGCTCCTCCGTCCAAATCCC
	<i>ZaARF9R</i>	CGACGTGAGTGACGAGGCAA
DN41058_c1_g1	<i>ZaARF10F</i>	CCCACCTTTACTACTGGCTTTATGG
	<i>ZaARF10R</i>	CCCAAGCGCTGGATTGAGA
DN41058_c1_g3	<i>ZaARF11F</i>	GGCTGGAGGATCACCTACCAAGTTA
	<i>ZaARF11R</i>	CGGCAGCTCTACGAGGAACA
DN41095_c1_g2	<i>ZaARF12F</i>	TCGGCGTCTTCAGCTTTCTGG
	<i>ZaARF12R</i>	CCATCAGCGTCTTTCTGCCTCA
DN42082_c1_g1	<i>ZaARF13F</i>	CCACGAGGTTCCGAGTGGAAG
	<i>ZaARF13R</i>	AGAGAATCCTGATGCAGCTCACAC
DN42082_c1_g2	<i>ZaARF14F</i>	GCCTAGCAGATTCTCAGGCTCTC
	<i>ZaARF14R</i>	CCTCACAGGAGGAACTACTGGCT
DN42234_c3_g3	<i>ZaARF15F</i>	TCAGCAGCAGCTTGAACGTACT
	<i>ZaARF15R</i>	GGGTGTCTTTACGTCGTTGTTATCCT
DN42397_c1_g1	<i>ZaARF16F</i>	TCCTGCCTCAGGTTGGGAGT
	<i>ZaARF16R</i>	CTTGGCACAGTAGTTGAAATGGGAGA
DN42397_c1_g2	<i>ZaARF17F</i>	TGCCAAATGAGCCTTCAACCAGT
	<i>ZaARF17R</i>	TGTGTGCTTGTGTGCTTGC
DN42397_c1_g4	<i>ZaARF18F</i>	TATGGCATGCCTGTGCTGGT
	<i>ZaARF18R</i>	TGGGAATTTGCGAGGTTGCTGA
DN42397_c2_g1	<i>ZaARF19F</i>	AAGTCCGTGTTTGCACCCA

	<i>ZaARF19R</i>	CACTTGGAACCAGGCCACCT
DN36887_c1_g5	<i>ZaGA20ox1F</i>	TGCTTGACAGAGCAGTGGT
	<i>ZaGA20ox1R</i>	GCCATGCGAAGTCCGGATACA
	<i>ZaGA20ox2F</i>	AGCCAAGTGCTAATGCACCAGT
DN37407_c0_g1	<i>ZaGA20ox2R</i>	AGAACCCATGCCTTCGGCAA
	<i>ZaGA2ox1F</i>	CCTGAAGCCCTCGTTGTCAACAT
DN31732_c1_g1	<i>ZaGA2ox1R</i>	TGCAACCGAGAACCTCTCCAC
	<i>ZaGA2ox2F</i>	TGGATCCCAGTGGCTCCTGA
DN34211_c0_g1	<i>ZaGA2ox2R</i>	CGCCATTGACATTCTCGACTTGC
	<i>ZaGA2ox4F</i>	CAACGTTGGTGATGCCTTGACAG
DN34416_c0_g2	<i>ZaGA2ox4R</i>	CACTCAAAGGTGGCCCTCCA
	<i>ZaGID1F</i>	ACCGCAATTGGTACTGGAGAGC
DN28368_c1_g1	<i>ZaGID1R</i>	TCCAAACCAGCTACAGAGACGAGA
	<i>ZaDELLA1F</i>	GCGAGCAACGCCACATTCAG
DN31406_c0_g1	<i>ZaDELLA1R</i>	AGGACTCAGCAAAGCTTGCCA
	<i>ZaDELLA2F</i>	TGTGGCGGTAGTGGAAATGGA
DN31914_c0_g3	<i>ZaDELLA2R</i>	CAGAACTCCAATAACTCGTCCATGC
	<i>ZaDELLA3F</i>	TGGCGGCATGGACGAGTTAC
DN38775_c0_g5	<i>ZaDELLA3R</i>	AGCAGAACCCATCACCATCTCG
	<i>ZaDELLA4F</i>	AGCATCACAAGCCGGTGCTA
DN40336_c2_g3	<i>ZaDELLA4R</i>	ACGGGCAGGTCTCGTAGAAGT
	<i>ZaDELLA5F</i>	GAGCTCCGACAAGTGGCTCA
DN40369_c1_g4	<i>ZaDELLA5R</i>	CCAAGAGGCGAGATCAGAGGGA
	<i>ZaDELLA6F</i>	AGGCAAGCCAGTATGTTGTTGACT
DN40369_c1_g5	<i>ZaDELLA6R</i>	CCAAGCCGAAGCCGCTATGA

Results

*Morphological and anatomical characteristics of fruits in two varieties of *Zanthoxylum armatum**

The floral and fruit morphology of ‘Jiuyeqing’ and ‘Prickleless’ were characterized using stereomicroscopic observation and histological sectioning (Figure 1). Both varieties exhibited dioecious, with cultivated strains being purely female and demonstrating apomictic traits. The female flowers of ‘Jiuyeqing’ consisted of only two whorls of floral organs (perianth and pistil), appearing pale green and approximately 2-3 mm in length at initial blooming with undivided styles (Figure 1A, B). Following with development, the flowers elongated to 3-4 mm, turning pale yellowish-green and assuming a vase-shaped morphology. The pistil is composed of two separate carpels, with forked pale yellowish-green stigmas at the tips of the carpels (Figure 1C, D). Subsequently, ovary enlargement led to fruit development, with individual fruit reaching about 2 mm in diameter while maintaining light green coloration (Figure 1E, F). The ‘Prickleless’ cv. displayed similar female floral morphology across all developmental stages (Figure 1G-L). Anatomical examination of pistils at different developmental stages revealed that each ovary contains two ovules. At the stage 1(S1), the ovary began to swell with megaspore mitosis forming mononuclear embryo sacs at the chalazal end, accompanied by degeneration phenomena (Figure. 1M). At the stage 2(S2), mature embryo sacs formed with polar nuclei developing into endosperm (Figure. 1N). While at the stage 3 (S3), the ovules became translucent with dense cytoplasmic nucellar cells, typically showing asynchronous development where one ovule became dominant while the other exhibited delayed growth and eventual degeneration (Figure. 1O). Notably, despite the

morphological differences between the two varieties, they exhibit similar patterns during ovary and ovule development.

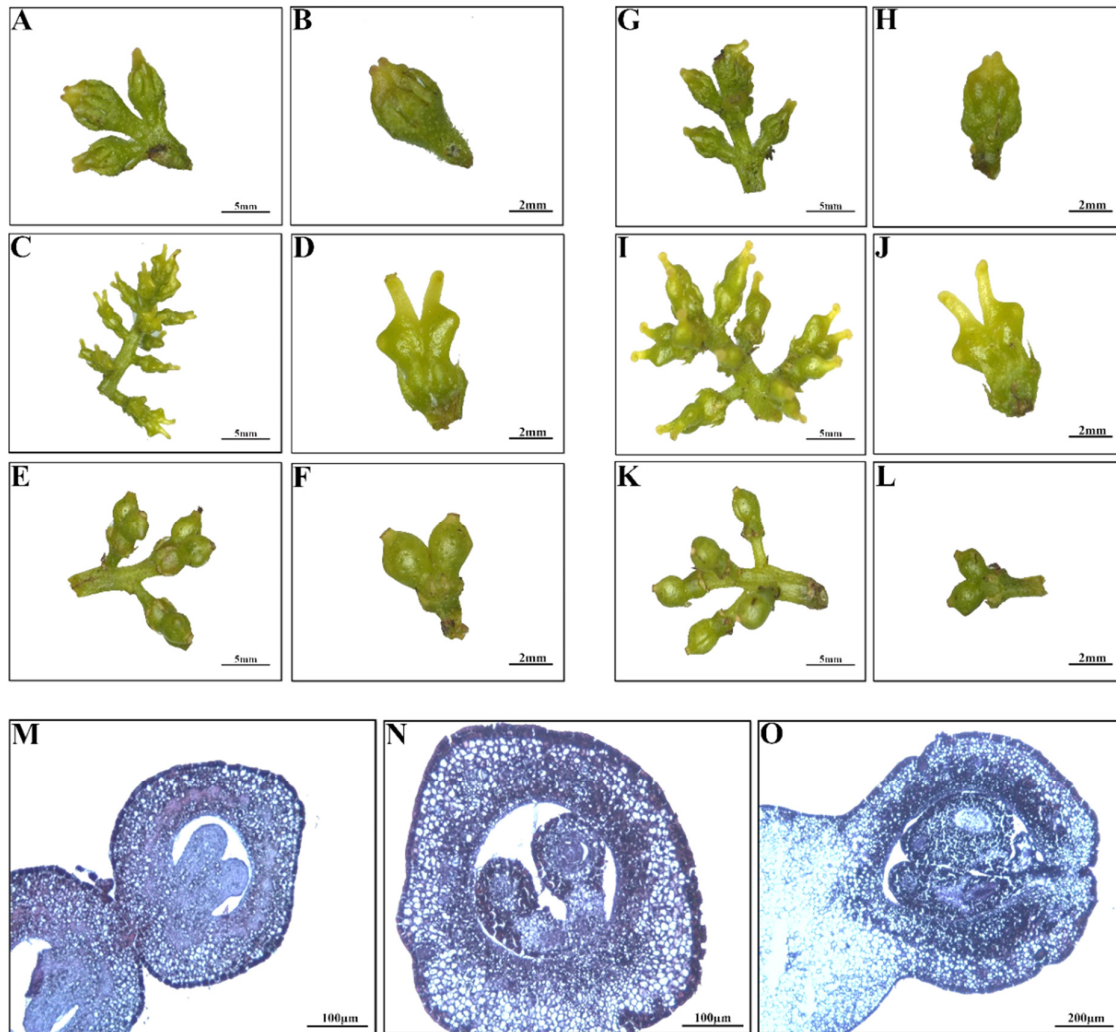


Figure 1. Fruit morphology and anatomical characteristics of *Z. armatum*. (A) and (B) represent female flowers of *Z. armatum* cv 'Jiuyeqing' at the ovule primordium formation stage (S1). (C) and (D) represent the ovule formation stage (S2). (E) and (F) represent the ovule enlargement stage (S3). (G) and (H) represent female flowers of 'Prickleless' cv. at the ovule primordium formation stage (S1), (I) and (J) illustrate the ovule formation stage (S2), and (K) and (L) represent PL at the ovule enlargement stage (S3). (M), (N) and (O) indicate anatomical structures of ovaries at three developmental stages, S1, S2, and S3, respectively

Identification of fruit-setting related genes in Zanthoxylum armatum

Previous studies showed that successful fruit setting requires the coordinated action of multiple hormones, among which auxin and GA are key regulatory factors. The auxin early response gene family Aux/IAA and auxin response factors (ARFs) constitute the core components of auxin signaling pathways. Transcriptomic analysis of female and male flowers in 'Jiuyeqing' identified differentially expressed auxin signaling genes, including 21 *Aux/IAA* genes (designated *ZaLAA1-ZaLAA21*), among which 19 *ZaLAAs* showed higher expression in female flowers compared to male flowers, except for *ZaLAA8* and *ZaLAA21*.

Additionally, 19 ARF genes (*ZaARF1-ZaARF19*) with differential expression between male and female flowers were identified, among which 10 *ZaARFs* exhibited significantly higher expression in female flowers (Table 2). Regarding GA metabolism and signaling components, we identified 2 *GA20ox* genes (*ZaGA20ox1-2*), 3 *GA2OX* genes (*ZaGA2OX1/2/4*), 1 *GIDI* gene (*ZaGIDI*), and 6 *DELLA* genes (*ZaDELLA1-6*) showing differential expression between female and male flowers (Table 3). These findings suggest that these differentially expressed genes involved in auxin and GA biosynthesis/signaling may play potential roles in fruit setting of *Zanthoxylum*.

Table 2. DEGs involved in auxin signal transduction between female and male flowers of *Zanthoxylum armatum*

Gene name	FF_counts	MF_counts	Log2FC (MF/FF)	Regulate	Gene annotation
<i>ZaIAA1</i>	1199.51	640	-1.14	down	<i>AtIAA18</i>
<i>ZaIAA2</i>	4098.49	2345	-1.04	down	<i>AtIAA18</i>
<i>ZaIAA3</i>	12.09	5.12	-1.47	down	<i>AtIAA27</i>
<i>ZaIAA4</i>	716.91	287.88	-1.55	down	<i>AtIAA27</i>
<i>ZaIAA5</i>	2230.02	1148.21	-1.19	down	<i>AtIAA9</i>
<i>ZaIAA6</i>	133	48	-1.7	down	<i>AtIAA13</i>
<i>ZaIAA7</i>	202	60	-1.98	down	<i>AtIAA13</i>
<i>ZaIAA8</i>	20	178	2.92	up	<i>AtIAA33</i>
<i>ZaIAA9</i>	206	120	-1.01	down	<i>AtIAA18</i>
<i>ZaIAA10</i>	167	76	-1.37	down	<i>AtIAA18</i>
<i>ZaIAA11</i>	126	35	-2.08	down	<i>AtIAA19</i>
<i>ZaIAA12</i>	38	14	-1.67	down	<i>AtIAA26</i>
<i>ZaIAA13</i>	1811	928	-1.19	down	<i>AtIAA26</i>
<i>ZaIAA14</i>	39	14	-1.71	down	<i>AtIAA7</i>
<i>ZaIAA15</i>	29	13	-1.39	down	<i>AtIAA16</i>
<i>ZaIAA16</i>	274.5	110	-1.55	down	<i>AtIAA16</i>
<i>ZaIAA17</i>	257	108	-1.48	down	<i>AtIAA16</i>
<i>ZaIAA18</i>	3915	2134	-1.11	down	<i>AtIAA16</i>
<i>ZaIAA19</i>	162	52	-1.87	down	<i>AtIAA16</i>
<i>ZaIAA20</i>	1040	425	-1.52	down	<i>AtIAA29</i>
<i>ZaIAA21</i>	9	27	1.35	up	<i>AtIAA16</i>
<i>ZaARF1</i>	693	293	-1.47	down	<i>AtARF16</i>
<i>ZaARF2</i>	40.97	11	-2.13	down	<i>AtARF2</i>
<i>ZaARF3</i>	1723.15	6403.68	1.66	up	<i>AtIAA24</i>
<i>ZaARF4</i>	37	585.37	3.75	up	<i>AtARF5</i>
<i>ZaARF5</i>	1535	764.47	-1.24	down	<i>AtARF4</i>
<i>ZaARF6</i>	1731.86	784	-1.37	down	<i>AtARF2</i>
<i>ZaARF7</i>	733.14	327	-1.4	down	<i>AtARF2</i>
<i>ZaARF8</i>	34	12	-1.73	down	<i>AtARF2</i>
<i>ZaARF9</i>	301	872	1.3	up	<i>AtARF17</i>
<i>ZaARF10</i>	1078	569.53	-1.15	down	<i>AtARF17</i>
<i>ZaARF11</i>	399	232	-1.01	down	<i>AtARF4</i>
<i>ZaARF12</i>	664.12	327.09	-1.25	down	<i>AtARF18</i>
<i>ZaARF13</i>	40	679.37	3.86	up	<i>AtARF5</i>
<i>ZaARF14</i>	485.89	1850.56	1.7	up	<i>AtARF5</i>

<i>ZaARF15</i>	29	14	-1.28	down	<i>AtARF10</i>
<i>ZaARF16</i>	11	341.31	4.73	up	<i>AtARF5</i>
<i>ZaARF17</i>	332	1114.6	1.52	up	<i>AtARF5</i>
<i>ZaARF18</i>	214	895.01	1.83	up	<i>AtARF5</i>
<i>ZaARF19</i>	49.02	1040.03	4.18	up	<i>AtARF5</i>

Table 3. DEGs involved in GA metabolism and signaling pathway between female and male flowers of *Zanthoxylum armatum*

Gene name	FF_counts	MF_counts	log2fc	Regulate	Gene annotation
<i>ZaGA20ox1</i>	15	307	4.12	up	<i>AtGA20ox1</i>
<i>ZaGA20ox2</i>	111	334.01	1.36	up	<i>AtGA20ox2</i>
<i>ZaGA2OX1</i>	412	55	-3.14	down	<i>AtGA2OX8</i>
<i>ZaGA2OX2</i>	133	24	-2.7	down	<i>AtGA2OX4</i>
<i>ZaGA2OX4</i>	17	116	2.54	up	<i>AtGA2OX2</i>
<i>ZaGID1</i>	481.91	241	-1.23	down	<i>AtGID1b</i>
<i>ZaDELLA1</i>	84	262	1.41	up	<i>AtGAI</i>
<i>ZaDELLA2</i>	7.02	19.04	1.21	up	<i>AtRGL1</i>
<i>ZaDELLA3</i>	19	49	1.14	up	<i>AtRGL1</i>
<i>ZaDELLA4</i>	76	238	1.42	up	<i>AtRGL1</i>
<i>ZaDELLA5</i>	89	42	-1.31	down	<i>AtRGL1</i>
<i>ZaDELLA6</i>	389	171.56	-1.41	down	<i>AtRGA</i>

Phylogenetic relationships of fruit-setting related genes in Zanthoxylum armatum

A phylogenetic tree was constructed using 21 *Aux/LAA* genes identified in *Z. armatum* along with 18 *Aux/LAA* homologs in *Arabidopsis* (Figure 2A). According to previous classification criteria (Remington *et al.*, 2004), the *Aux/LAA* genes in *Z. armatum* were systematically divided into two major clades (A and B), with clade A further divided into four subgroups (A1-A4) and clade B into three subgroups (B1-B3). The A1 subgroup contained a single *ZaLAA* gene (*ZaLAA16*), which was clustered together with *AtLAA7*, *AtLAA14* and *ZaLAA17*. Three *ZaLAAs* (*ZaLAA18*, *ZaLAA19*, *ZaLAA21*) belonged to the A2 subgroup, showing high homology with *AtLAA16*. The A3 subgroup comprised three genes (*ZaLAA3*, *ZaLAA4*, *ZaLAA5*) that grouped with *AtLAA8*, *AtLAA9* and *AtLAA17*. Six *ZaLAAs* (*ZaLAA1*, *ZaLAA11*, *ZaLAA13*, *ZaLAA14*, *ZaLAA15*, *ZaLAA17*) constituted the A4 subgroup and clustered with *AtLAA5*, *AtLAA6* and *AtLAA19*. The B clade contained two genes, *ZaLAA8* and *ZaLAA20* in subgroup B1 that were homologous to *AtLAA29*, while subgroup B2 included two genes, *ZaLAA6* and *ZaLAA7*, which showed homology to *AtLAA12*, *AtLAA13* and *AtLAA32*. Finally, subgroup B3 comprised four genes, including *ZaLAA2*, *ZaLAA9*, *ZaLAA10*, *ZaLAA12*, that formed a distinct cluster with *AtLAA18* and *AtLAA26*.

To investigate the evolutionary relationships among *ZaARF* proteins, we constructed a phylogenetic tree comprising the ARF protein families from *Zanthoxylum* and *Arabidopsis* (Figure 2B). According to the tree topology, the 19 *ZaARF* proteins were classified into four distinct subfamilies (Class I-IV). Class I represented the largest subgroup containing 14 members (*ZaARF1-8*, *ZaARF10*, *ZaARF12-15*, and *ZaARF19*). Class II comprised three *ZaARFs* (*ZaARF16-18*) that clustered together with *AtARF5*, *ZaARF7*, and *AtARF19*. Class III contained a single member *ZaARF11*, which showing homology to *AtARF3* and *AtARF4*. The smallest subgroup, Class IV, included *ZaARF9*, which was phylogenetically related to *AtARF10*, *AtARF16*, and *AtARF17*.

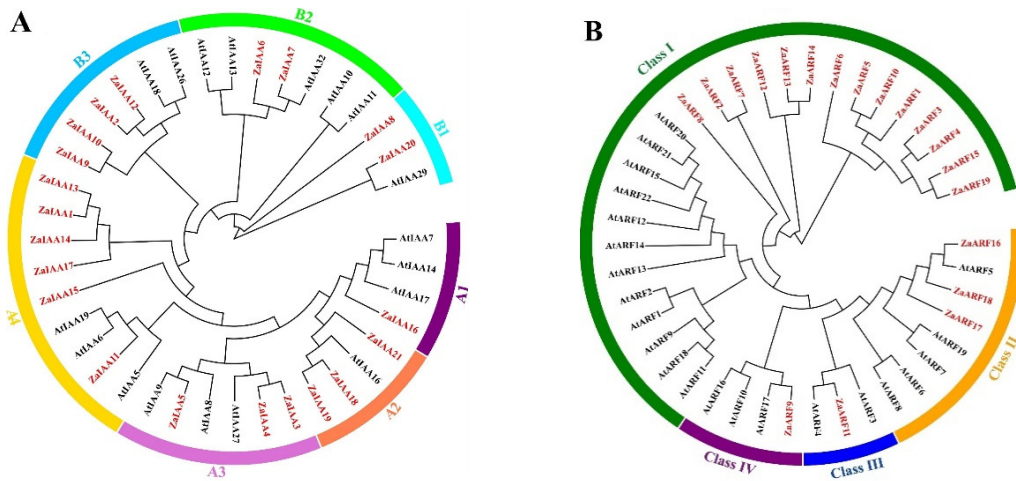


Figure 2. Phylogenetic analysis of auxin signaling genes: (A) The Aux/IAA proteins in *Z. armatum* and *Arabidopsis thaliana* were divided into two groups, group A and B were divided into 4 and 3 subgroups (A1~A4, B1~B3), respectively. (B) ARF proteins in *Z. armatum* and *Arabidopsis* can be divided into 4 subfamilies (I~IV)

Phylogenetic analysis of GA biosynthesis and signaling-related genes revealed distinct evolutionary relationships among *Zanthoxylum* homologs (Figure 3A-D). For *GA20-oxidase* genes, *ZaGA20ox1* showed high homology with cucumber *CcGA20ox1D* and *CcGA20ox2*, while *ZaGA20ox2* clustered with tomato *SlGA20ox1* and citrus *CsGA20ox1D* (Figure 3A). Regarding *GA2-oxidases*, *ZaGA2OX1* and *ZaGA2OX4* were phylogenetically closest to *Arabidopsis AtGA2OX2* and tomato *SlGA2OX1*, whereas *ZaGA2OX2* formed a distinct clade with grape *VvGA2OX4* and cucumber *CcGA2OX2/4* (Figure 3B). The GA receptor *ZaGID1* exhibited highest similarity to cucumber *CcGID1b* (Figure 3C). Among DELLA proteins, *ZaDELLA1* was grouped with cucumber *CcRGL1*, citrus *CsRGL2*, tomato *SlRGL1*, and grape *VvRGL1/VvGAI*. *ZaDELLA2-4* showed strong homology to citrus *CsRGA*, tomato *SlGAI*, and grape *VvDELLA2*. *ZaDELLA5/6* were most closely related to cucumber *CcGAI* (Figure 3D).

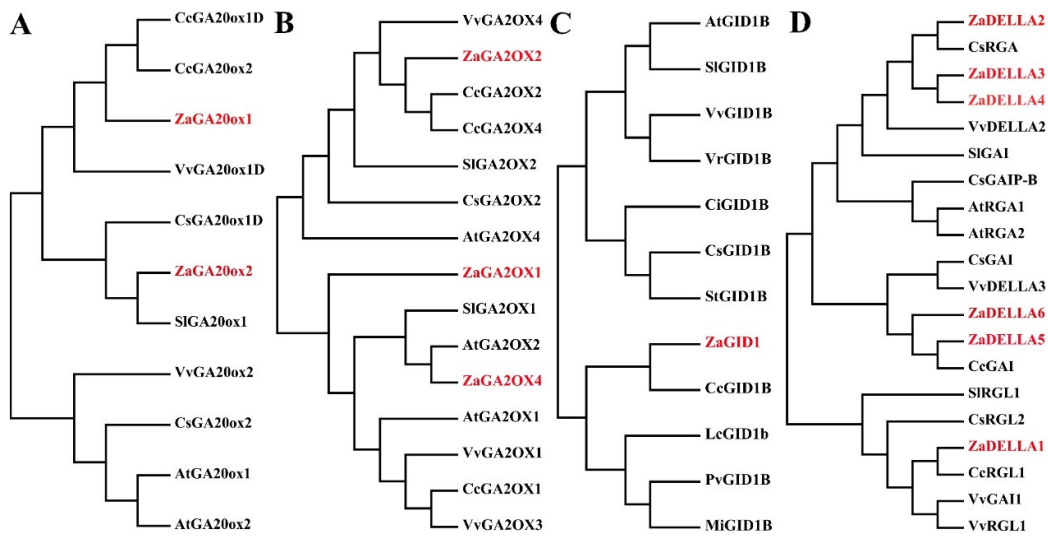
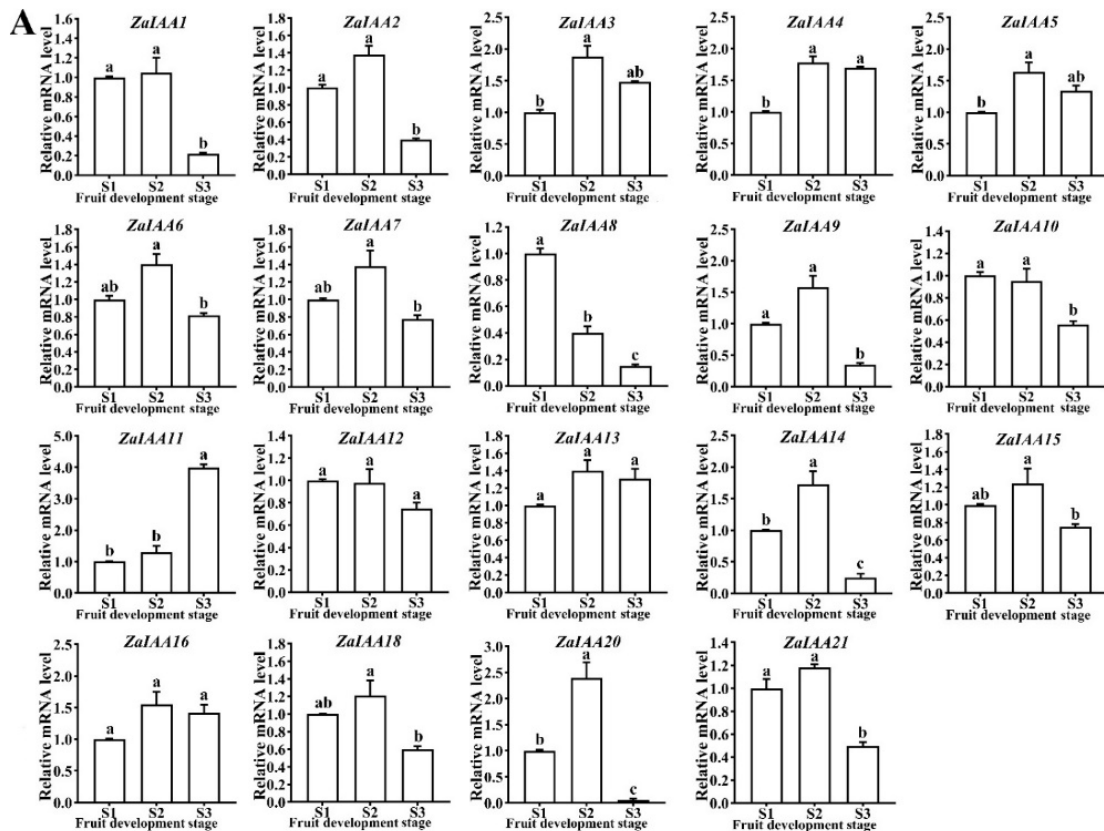


Figure 3. Phylogenetic analysis of GA biosynthesis and signaling related genes

Expression profiles of genes associated with auxin signaling pathway during fruit set in Z. armatum

To investigate the potential roles of auxin signaling genes during *Z. armatum* fruit development, we analyzed the expression patterns of 21 *ZaLAAs* and 19 *ZaARFs* using qRT-PCR. In cultivar ‘Jiuyeqing’, several *ZaLAAs* including *ZaLAA3*, *ZaLAA4*, *ZaLAA5*, *ZaLAA14* and *ZaLAA20*, showed significant upregulation of their expressions during pistil development (S1 to S2). Subsequently, during the fruit formation stage (S2 to S3), the mRNA levels of *ZaLAA14* and *ZaLAA20* were decreased, while the expressions of the other genes remained stable. In contrast, the expression levels of *ZaLAA1*, *ZaLAA2*, *ZaLAA9*, *ZaLAA15*, *ZaLAA18* and *ZaLAA21* showed a downward trend throughout the fruit development process, while *ZaLAA11* and *ZaLAA8* displayed opposite patterns with increasing and decreasing expression during fruit set and development respectively. The remaining *ZaLAA* genes showed no significant expression changes (Figure 4A). Regarding the *ARF* genes, *ZaARF1*, *ZaARF8*, *ZaARF10*, *ZaARF12* and *ZaARF15* were markedly upregulated during pistil development, followed by either decreased expression or stable levels (*ZaARF1*) in subsequent stages. During fruit formation, 11 *ZaARF* genes (*ZaARF2* to *ZaARF5*, *ZaARF7*, *ZaARF11*, *ZaARF13*, *ZaARF16* to *ZaARF19*) displayed remarkable downregulation (Figure 4B).



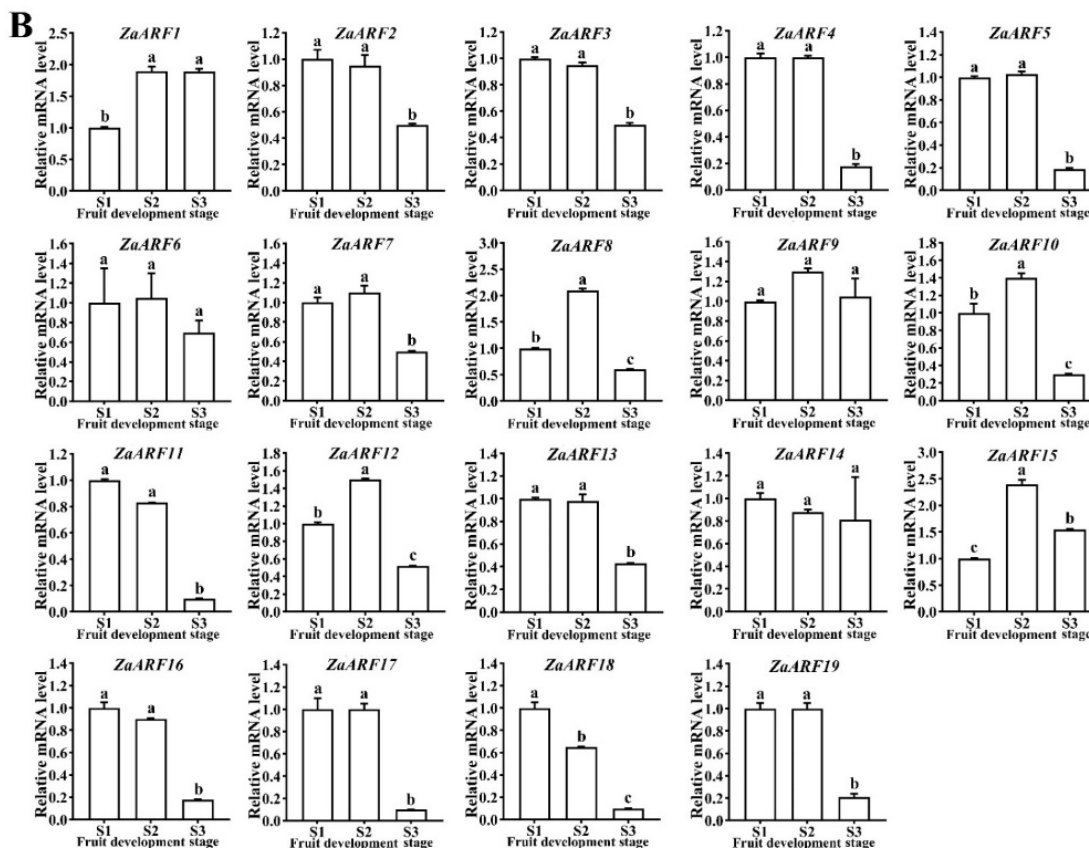


Figure 4. Expression levels of auxin signaling genes during fruit set in *Z. armatum* cv 'Jiuyeqing'. Real-time quantitative PCR (qRT-PCR) was employed to analyze the expression patterns of auxin signaling related genes, including Aux/IAAs (A) and ARFs (B), during the fruit set process in cv 'Jiuyeqing'. Relative expression levels were normalized to the S1 control (set as 1)

Three biological replications were performed for each examination, Duncan's multiple range test was used to analyze the significance, and the different lowercase letters indicate significant ($P < 0.05$) differences between samples

In the 'Prickleless' cv., five *Aux/IAA* genes (*ZaIAA2*, *ZaIAA6*, *ZaIAA7*, *ZaIAA11*, and *ZaIAA15*) exhibited notably upregulation throughout pistil development and fruit formation, while *ZaIAA8* and *ZaIAA20* showed declined expression patterns during fruit setting. The remaining *ZaIAAs* demonstrated no obvious expression changes (Figure 5A). For *ARF* genes, the transcription levels of six members (*ZaARF1*, *ZaARF3*, *ZaARF8*, *ZaARF9*, *ZaARF18*, and *ZaARF19*) were gradually increased during pistil development and fruit formation. In contrast, *ZaARF5* and *ZaARF10* showed gradually decreasing mRNA abundance during fruit development. Notably, four *ARFs*, including *ZaARF2*, *ZaARF6*, *ZaARF13*, and *ZaARF14*, were significantly upregulated in young fruits compared to those in ovaries (Figure 5B).

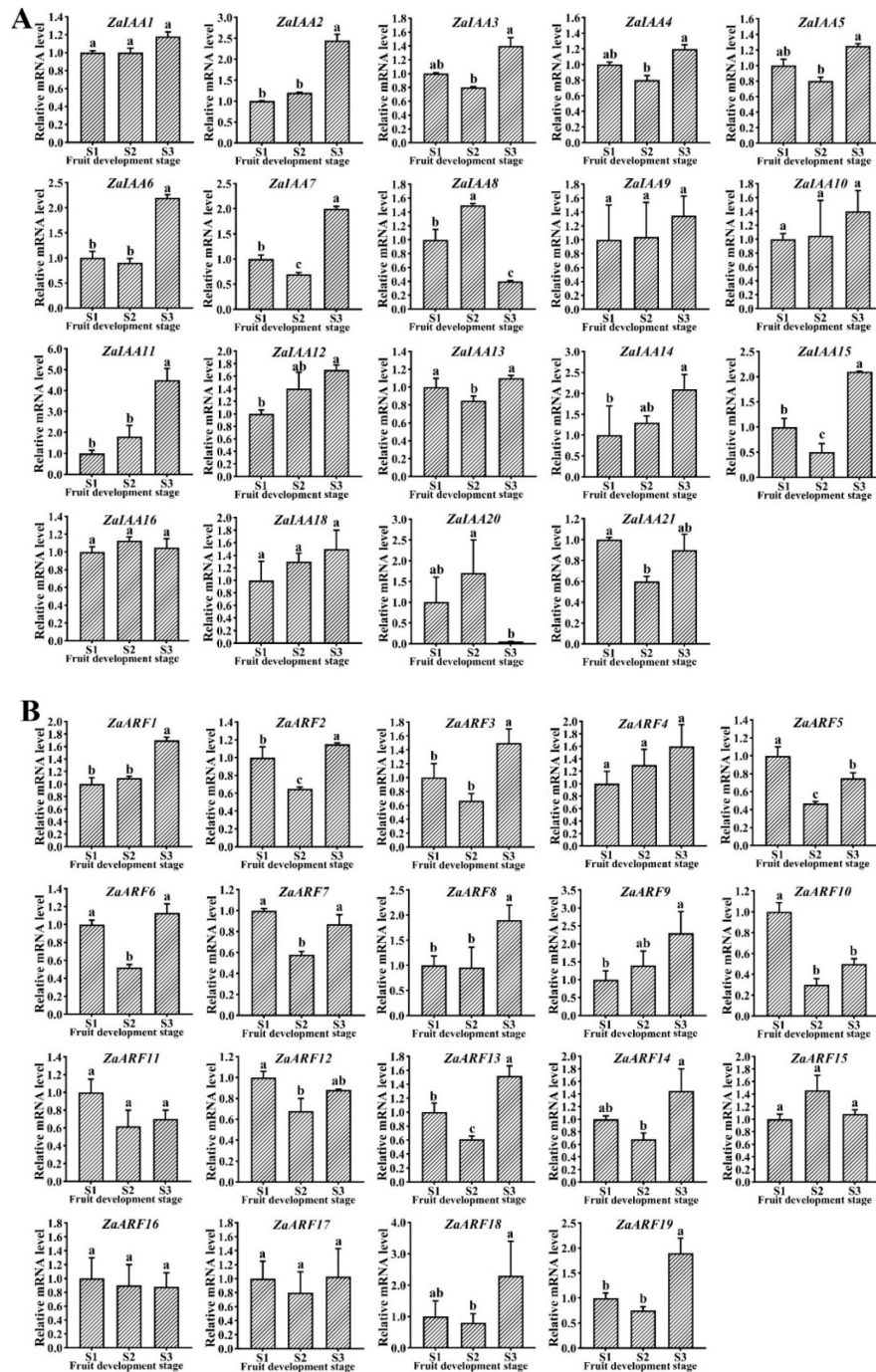


Figure 5. Expression profiles of auxin signaling related genes during fruit set in *Z. armatum*. cv. 'Prickless' QRT-PCR was employed to analyze the expression patterns of auxin signaling pathway related genes, including *Aux/IAAs* (A) and *ARFs* (B), during the fruit set process

Three biological replications were performed for each examination, Duncan's multiple range test was used to analyze the significance, and the different lowercase letters indicate significant ($P < 0.05$) differences between samples

Comparative analysis revealed three *Aux/IAA* genes (*ZaIAA8*, *ZaIAA11*, and *ZaIAA20*) and three *ARF* genes (*ZaARF1*, *ZaARF5*, and *ZaARF10*) exhibiting consistent expression trends during fruit development in

both ‘Jiuyeqing’ and ‘Prickleless’ cultivars. Specifically, *ZaLAA11* showed upregulated expression, while *ZaLAA8* and *ZaLAA20* were downregulated following fruits initiation. Similarly, *ZaARF1* expression was increased, whereas *ZaARF5* and *ZaARF10* were decreased across both cultivars. These conserved patterns suggest that these six genes, including *ZaLAA8*, *ZaLAA11*, *ZaLAA20*, *ZaARF1*, *ZaARF5*, and *ZaARF10*, might play fundamental roles in regulating fruit setting and development in *Zanthoxylum*.

Expression profiles of GA related genes in two cultivars of *Z. armatum*

Gibberellins play crucial regulatory roles during fruit development, as evidenced by expression analysis of 2 *GA20ox*, 3 *GA2OX*, 1 *GID1*, and 6 *DELLA* genes across different developmental stages in *Z. armatum* cv ‘Jiuyeqing’ (Figure 6). Gibberellin 20-oxidase (GA20ox) is a key enzyme for gibberellin biosynthesis. During pistil development and fruit formation, the expression levels of *ZaGA20ox1* and *ZaGA20ox2* were remarkably decreased (Figure 6A). Among the gibberellin catabolic genes, *ZaGA2OX1* was upregulated during fruit setting, while *ZaGA2OX4* exhibited the opposite trend. *ZaGA2OX2* displayed initial upregulation during pistil development followed by marked downregulation in fruit formation (Figure 6B). The mRNA level of GA receptor gene *ZaGID1* was gradually decreased during fruit setting (Figure 6C). DELLA proteins are negative regulators of GA signaling. The transcript levels of *ZaDELLA1* and *ZaDELLA3* were decreased during pistil and fruit development, while *ZaDELLA5* and *ZaDELLA6* showed an upward trend. The remaining *DELLAs* maintained stable expression (Figure 6D). These results suggest that differentially expressed GA metabolism and signaling genes, particularly *ZaGA2OX2*, *ZaGA2OX4*, *ZaDELLA1*, and *ZaDELLA3*, may play crucial regulatory roles in the fruit setting process of ‘Jiuyeqing’ cv. We further investigated the expression patterns of *GA20ox*, *GA2OX*, *GID1*, and *DELLA* genes during fruit development in ‘Prickleless’ cv. (Figure 7). The mRNA level of *ZaGA20ox2* was significantly decreased during pistil development but then sharply increased following with fruit formation (Figure 7A). Both *ZaGA2OX2* and *ZaGID1* showed gradually enhanced expression during fruit development (Figure 7B, C). For DELLA genes, the transcriptional level of *ZaDELLA1* was dramatically increased during pistil development but then decreased during fruit formation. *ZaDELLA5* and *ZaDELLA6* displayed significantly elevated expressions during fruit setting. The remaining genes displayed no notable expression changes (Figure 7D). These findings strongly suggest that *ZaGA20ox2*, *ZaGID1*, and *ZaDELLA1* might serve as key regulators mediating fruit setting in ‘Prickleless’ cultivar.

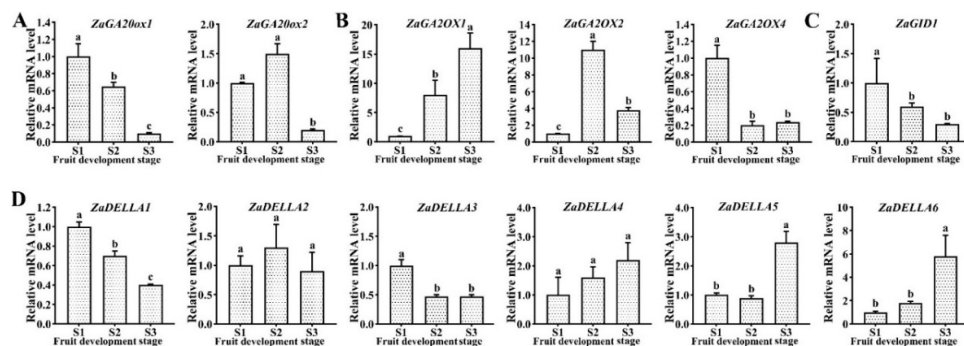


Figure 6. Expression levels of GA metabolism and signaling genes during fruit set in *Z. armatum* cv ‘Jiuyeqing’. Real-time quantitative PCR (qRT-PCR) was employed to analyze the expression patterns of gibberellin (GA) metabolism and signaling related genes during the fruit set process in cv ‘Jiuyeqing’, including GA biosynthesis genes *GA20oxs* (A), GA catabolism genes *GA2OXs* (B), GA receptor gene *GID1* (C), and negative regulatory factors *DELLAs* (D)

Three biological replications were performed for each examination, Duncan’s multiple range test was used to analyze the significance, and the different lowercase letters indicate significant ($P < 0.05$) differences between samples

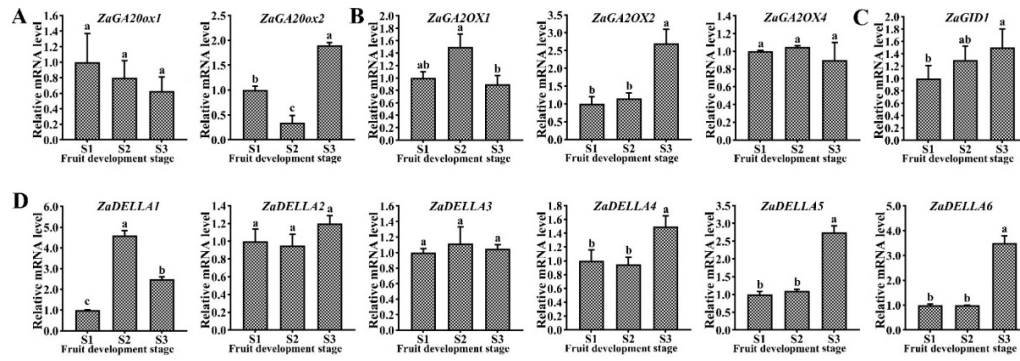


Figure 7. Expression levels of GA metabolism and signaling genes during fruit set in 'Prickleless' *Z. armatum*. Real-time quantitative PCR (qRT-PCR) was employed to analyze the expression patterns of gibberellin (GA) metabolism and signaling related genes during the fruit set process in 'Prickleless' cv., including GA biosynthesis genes *GA20oxs* (A), GA catabolism genes *GA20Xs* (B), GA receptor gene *GID1* (C), and negative regulatory factors *DELLAs* (D)

Three biological replications were performed for each examination, Duncan's multiple range test was used to analyze the significance, and the different lowercase letters indicate significant ($P < 0.05$) differences between samples

Discussion

Zanthoxylum armatum is one of the most distinctive mountainous economic tree species in Chongqing, China. However, severe physiological fruit drop and low fruit-setting rate during cultivation necessitate the application of plant growth regulators to improve yield. A comprehensive understanding of the molecular mechanisms underlying phytohormone-mediated fruit setting in *Z. armatum* is crucial for enhancing both productivity and fruit quality, providing essential theoretical foundations for sustainable cultivation.

Mechanism of auxin signaling genes in regulating fruit-setting of Zanthoxylum armatum

Auxin, a pivotal phytohormone, orchestrates diverse aspects of plant growth and development, including floral organ formation, fruit development, cell division, growth differentiation, and tropic responses (Enders *et al.*, 2015). The auxin signaling cascade is primarily governed by two transcriptional regulators, Aux/IAA repressors and ARF activators. Under low auxin conditions, Aux/IAA proteins dimerize with ARFs, inhibiting their transcriptional activation of downstream auxin-responsive genes. Elevated auxin levels promote TIR1/AFB receptor-mediated ubiquitination and 26S proteasomal degradation of Aux/IAAs, thereby releasing ARFs to activate auxin-responsive gene expression (Lakehal *et al.*, 2019). In this study, we identified 21 *Aux/IAA* genes differentially expressed in female and male flowers of cv 'Jiuyeqing' by using transcriptome analysis, suggesting their potential roles in fruit setting and apomixis. Notably, *ZaIAA3*, *ZaIAA4*, *ZaIAA5*, *ZaIAA14*, and *ZaIAA20* were upregulated during pistil development (Figure 4A), implicating their involvement in gynoecium maturation. Phylogenetic conservation underscores functional parallels. silencing of the *SILAA2* gene in tomatoes, which is homologous to *ZaIAA3/4*, enhances auxin sensitivity but impairs the fertility of ovules/pollen and alters the anatomy of flowers/fruit (Bassa *et al.*, 2012). Knockout of the *SILAA9* gene, which is homologous to *ZaIAA5*, induces parthenocarpy (Mazzucato *et al.*, 2015). Conversely, *ZaIAA1*, *ZaIAA2*, *ZaIAA8*, *ZaIAA9*, *ZaIAA14*, *ZaIAA20*, and *ZaIAA21* were downregulated during fruit formation, while *ZaIAA1* was upregulated, collectively highlighting their regulatory significance in fruit setting. *ZaIAA1/2/9* homolog in *Arabidopsis*, *AtLAA18*, modulated embryonic patterning by suppressing *ARF5* (Luo *et al.*, 2018). Overexpression of *PIIAA19* in tomato, *ZaIAA11* homolog in peach, can trigger parthenocarpy and fruit elongation (Ding *et al.*, 2019). Divergent regulation was observed

in ‘prickleless’ cv. (PL), where *ZaLAA6/7* expression surged during fruit development (Figure 5A), mirroring their tomato homolog *SLIAA13*, which is induced post-pollination or auxin treatment (Tang *et al.*, 2017). Notably, except for *ZaLAA11* and *ZaLAA20*, auxin signaling dynamics markedly differed between cv ‘Jiuyeqing’ and ‘Prickleless’ during pistil and fruit development, contributing to PL’s precocious flowering and fruit-setting.

RNA-Seq analysis revealed 19 *ARF* genes differentially expressed between female and male flowers, implying their candidate roles in controlling pistil development and fruit setting. In the ‘Jiuyeqing’ cv., *ZaARF1*, *ZaARF8*, and *ZaARF15* were significantly upregulated during pistil development, while 12 *ARFs* (*ZaARF2-5*, *ZaARF7*, *ZaARF10-13*, *ZaARF16-19*) showed marked downregulation during fruit formation. In *Arabidopsis*, *AtARF3*, clustering with *ZaARF11*, mediates self-incompatibility signaling and pistil development interactions (Tantikanjana *et al.*, 2012). *ZaARF12* homolog *ChARF3* in hazelnut regulates ovule development via auxin biosynthesis/transport and cell proliferation pathways (Wei *et al.*, 2021). In tomato, *ZaARF16-18* ortholog *SLARF5* can promote cell division while inhibit expansion, with its suppression reducing pericarp cell layers but increasing cell volume. Notably, *SLARF5* interacts with *SLIAA9* and *SLARF7/8A/8B* to inhibit fruit set by activating MADS-box genes (*SLAG1*, *SLAGL6*, *SLMADS2*) (Hu *et al.*, 2023; Li *et al.*, 2024). Additionally, *ZaARF2/7* homolog *SLARF2* mediates auxin-gibberellin crosstalk during pollination-dependent and parthenocarpic fruit set (Tang *et al.*, 2015). In contrast, during fruit setting, the expressions of *ZaARF3*, *ZaARF13*, *ZaARF18* and *ZaARF19* were upregulated in ‘Prickleless’ cv., implying a divergent pattern potentially underlying its early-fruitlet trait. Notably, *ZaARF9* showed progressive upregulation in ‘Prickleless’ but no change in ‘Jiuyeqing’ cultivar. *ZaARF9* is phylogenetically related to *AtARF17*, which is involved in pollen wall formation in *Arabidopsis* (Yang *et al.*, 2013). Suppression of *ZaARF9* homolog in cucumber, *CsARF10a*, led to apomixis, while its overexpression in tomato delays fruit ripening (Xu *et al.*, 2024). These findings indicate *ZaARF9* might be a key candidate regulating early fruit setting and apomixis in ‘Prickleless’ cv.

Mechanism of GA metabolism and signaling genes in modulating fruit-setting of Z. armatum

Gibberellins (GAs), a class of tetracyclic diterpenoid compounds, play pivotal roles in various plant developmental processes including seed germination, stem and root elongation, floral induction, and flower development, while also serving as critical determinants of fruit set and fruit development (Sun *et al.*, 2011). In agricultural production, GAs are commonly applied to enhance flowering, improve fruit setting rates, and elevate fruit quality (Ruan *et al.*, 2012). For instance, GA₃ treatment can induce parthenocarpic fruit set in unpollinated tomato pistils (Tang *et al.*, 2015). The biosynthesis of bioactive GAs (e.g., GA₃ and GA₄) is catalyzed by GA 20-oxidases (GA20oxs), whereas GA 2-oxidases (GA2oxs) mediate their deactivation. Overexpression of citrus *CcGA20ox1* in tomato promotes plant height and parthenocarpy (García-Hurtado *et al.*, 2012). Wang *et al.* (2020) identified three *PbGA20ox* genes (*PbGA20ox1-3*) in pear, with *PbGA20ox2* exhibiting high expression in young fruits and significant upregulation post-pollination. Transient expression of *PbGA20ox2* promotes fruit development and delays abscission in unpollinated fruits, while its stable overexpression in tomato results in elongated hypocotyls, extended internodes, and parthenocarpy upon emasculatation. In this study, candidate *ZaGA20ox* genes involved in fruit development were identified. QRT-PCR analysis revealed that *ZaGA20ox1* and *ZaGA20ox2* were downregulated during fruit set in cv. ‘Jiuyeqing’, whereas *ZaGA20ox2* showed marked upregulation in ‘Prickleless’ cv., suggesting its pivotal role in regulating fruit set and development. Research on GA catabolic enzymes demonstrates tissue-specific expression patterns. In *Arabidopsis*, *GA2ox1* is expressed in hypocotyls and lateral root primordia, *GA2ox2* in aerial tissues, *GA2ox3* in chalazal endosperm and inflorescences, *GA2ox4* in shoot apical meristems and lateral root initiation, *GA2ox6* in root maturation zones, *GA2ox7* constitutively across developmental stages, and *GA2ox8* exclusively

in guard cells (Li *et al.*, 2019). In tomato, silencing all five C19-class *GA2ox* genes elevates GA₄ levels in ovaries, resulting in an approximately 30-fold increase in the size of unpollinated ovaries and a parthenocarpy rate of 5% to 37% (Martínez-Bello *et al.*, 2015). Chen *et al.* (2016) reported that fruit-specific overexpression of *SlGA2ox1* reduces fruit weight and seed number, downregulates cell expansion and GA-responsive genes, indicating its role in modulating fruit and seed development via GA depletion. Our findings demonstrate that *ZaGA2ox2* is induced during pistil development but suppressed during fruit set in cv. 'Jiuyeqing', while *ZaGA2ox4* is downregulated throughout both stages. This negative correlation with fruit development implies their potential roles in regulating fruit set and apomixis by modulating bioactive GA levels.

Gibberellin Insensitive Dwarf1 (GID1), a nuclear-localized GA receptor, initiates growth promotion by perceiving GA and triggering degradation of DELLA repressors. This DELLA-GID1 regulatory module plays pivotal roles in fruit development. In *Arabidopsis*, three *GID1* genes were present and displayed distinct expression patterns, with *GID1A* throughout pistils, *GID1B* in ovules, and *GID1C* in fruit valves. Double mutants *gid1a gid1b* and *gid1a gid1c* exhibit reduced seed numbers and shorter siliques, demonstrating GID1's role in fruit set and growth (Gallego-Giraldo *et al.*, 2014). In cucumber, the expression pattern of *CsGID1a* was found to be closely correlated with fruit locule formation, and silencing *CsGID1a* in cucumber resulted in fruits with abnormal carpels and locules. Overexpression of *CsGID1a* in the *Arabidopsis* double mutant (*gid1a gid1c*) resulted in 'cucumber locule-like' fruits (Liu *et al.*, 2016). Similarly, GID1 regulates apomictic embryo sac formation in *Stylosanthes* (Ferreira *et al.*, 2018). In this study, *ZaGID1* (the *Arabidopsis* *GID1* ortholog) showed higher expression in female than male *Zanthoxylum* flowers and was upregulated during pistil development and fruit set in 'Prickleless' cv., suggesting its involvement in apomixis and fruit development. DELLA proteins also exhibit specialized functions. The DELLAs proteins in *Arabidopsis* control style/stigma growth (Fuentes *et al.*, 2012), among which RGA is ubiquitously expressed, GAI is valve-specific, RGL1 is expressed in the endocarp/pollinated placenta, and RGL2 is expressed in the ovules/seeds, displaying spatial expression patterns mirroring *GID1A-C* (Gallego-Giraldo *et al.*, 2014). In tomato, RNAi-mediated *SIDELLA* silencing upregulates GA biosynthesis genes and induces parthenocarpy, while wild-type ovaries abort when pollination is blocked due to elevated *SIDELLA* levels. The *proΔGRAS* DELLA mutant exhibits fertilization defects due to excessive style elongation but achieves parthenocarpy via sustained GA synthesis and metabolite accumulation (Ezura *et al.*, 2023). Our results revealed that *ZaDELLA1/3* (tomato *SIDELLA* homologs) are downregulated during pistil development and fruit set in cv. 'Jiuyeqing', consistent with their proposed role in apomictic fruit formation. Conversely, five *ZaDELLAs* are upregulated in cv. 'Prickleless', suggesting a potential GID1-mediated GA response pathway independent of DELLA repression (Fuentes *et al.*, 2012).

Conclusion

In summary, this study characterized the anatomical structures of ovary development and fruit formation in two *Zanthoxylum* cultivars with different maturation periods using histological sectioning. Through transcriptomic analysis, we identified candidate auxin- and gibberellin-related genes regulating fruit set. The expression profiles of these candidate genes during pistil development and fruit setting in both cultivars were investigated. By integrating functional studies of homologous genes, we identified key regulators of apomixis and fruit development in *Zanthoxylum*, including Aux/IAA (*ZaIAA1-ZaIAA6*, *ZaIAA9*, and *ZaIAA11*), ARF (*ZaARF2*, *ZaARF7*, *ZaARF9*, *ZaARF11*, *ZaARF12*, and *ZaARF16-ZaARF18*), and GA-related genes (*ZaGA2ox2*, *ZaGA2OX2*, *ZaGA2OX4*, *ZaGID1*, *ZaDELLA1*, and *ZaDELLA3*). However, the exact functions of these genes and the mechanisms by which they respond to hormone signals to regulate fruit development remain to be fully elucidated in further research.

Authors' Contributions

Conceptualization, NT and ZC; Methodology, NT and QL; Software, QL and SM; Validation, NT, ZC and QL; Formal analysis, ZL; Investigation, SM and ZL; Resources, QL and ZL; Data curation, QL and SM; Writing-original draft, QL; Writing-review and editing, ZC; Visualization, NT; supervision, ZC; Funding acquisition, ZC.

All authors have read and agreed to the published version of the manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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