

The Jasmonic Acid Signaling Pathway Plays an Important Role in *Ziziphus jujuba* Resistance to *Apolygus lucorum*

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Abstract

Apolygus lucorum is a polyphagous herbivore that has become a major pest in China. Jujube (*Ziziphus jujuba*) is the main host of *A. lucorum*. However, better insight into the molecular mechanisms underlying the jujube defense response to *A. lucorum* is needed. Here, we investigated the resistance of resistant (winter jujube) and sensitive varieties (wild jujube) to *A. lucorum*. Changes in the transcriptome, jasmonic acid (JA) and salicylic acid (SA) contents and the expression of genes involved in signaling pathways in different jujube varieties were monitored. The preference of *A. lucorum* for jujube with exogenous SA and methyl jasmonate (MeJA) was examined. Wild jujube showed more resistance and nonselectivity to *A. lucorum*. RNA-seq analysis showed that 438 and 796 transcripts were differentially expressed in winter jujube and wild jujube leaves infested by *A. lucorum*. Enrichment analysis showed that more genes in JA pathways were upregulated and more genes in SA pathways were downregulated. *A. lucorum* increased the JA content and decreased the salicylic acid content. This was confirmed by the alterations of gene expression in the related signaling pathways. MeJA in jujube leaves showed an obvious repellent effect on *A. lucorum*. Consistently, the contents of jasmonic acid and salicylic acid in winter jujube were lower than those in wild jujube leaves. The present data indicated that *A. lucorum* infestation of jujube plants induced the JA pathway and suppressed the SA pathway. JA in jujube leaves played an important role in repelling and basic defense against *A. lucorum*.

Key Messages

- Wild jujube showed more resistance and non-selectivity to *A. lucorum* than winter jujube.
- *A. lucorum* infestation induced JA pathway and suppressed SA pathway in jujube leaves.
- JA in jujube leaves played an important role in repellent and basic defense to *A. lucorum*.

Introduction

Chinese jujube (*Ziziphus jujuba*) is a tree originating from China that produces an economically important fruit that is widely grown in temperate regions of the Northern Hemisphere (Cheng et al. 2020; Liu et al. 2013). Jujube plants are frequently subject to attack by different types of pests, including *Apolygus lucorum* (Meyer-Dür), lepidopteran larvae, spider mites and mirids, such as the green plant bug (Pan et al. 2021; Zhen and Gao 2016).

In recent years, with the adjustment of the industrial structure and the increase in fruit and vegetable planting area, *A. lucorum* has become a major pest in China (Lu et al. 2010). *A. lucorum* is a polyphagous herbivore. It can feed on 288 host plants from 54 families (Pan et al. 2015a). As the major overwintering host of *A. lucorum*, jujube leaves began to germinate when the overwintering eggs of *A. lucorum* hatch, resulting in serious damage to the new leaves of jujube trees. In China, the damage rate of jujube leaves caused by *A. lucorum* has risen from 20%~30% in 1997 to 80%~90% now, and the extent of damage is increasing year by year.

Herbivorous insect feeding and infection with plant pathogens can induce plant defense responses. The terminal products of the signal response pathway can activate the expression of defense genes in plants so that the plants show a resistance response (Erb and Reymond 2019). The two major defense signaling pathways in plants are the jasmonic acid (JA)- and salicylic acid (SA)-mediated pathways (Erb and Reymond 2019; Howe et al. 2018). Different feeding patterns of insects induce different types of plant defense responses (Davila Olivas et al. 2017). Generally, chewing herbivores and necrotrophic pathogens usually elicit jasmonic acid (JA)-mediated plant defenses (Moran and Thompson 2001), while the salicylic acid (SA)-responsive pathway modulates defenses against piercing/sucking insects and biotrophic pathogens (Moran and Thompson 2001; Stout et al. 1999). Even when herbivores have the same feeding mode, the induced defense response of the host plants can be significantly different. For example, the JA and SA signaling pathways were both induced by *Myzus persicae* feeding (Moran and Thompson 2001), while silverleaf whiteflies feeding on *Arabidopsis thaliana* induced the SA defense pathways and inhibited the JA defense pathways (Louisa A. Kempema 2007).

A. lucorum is usually classified with phloem-feeding insects as piercing/sucking herbivores. However, they use stylets to vigorously puncture plant cells. Therefore, their feeding causes more intense physical damage than most phloem-feeding insects (Backus et al. 2007). Studies have shown that the activity of defense enzymes increases after *A. lucorum* infestation of cotton, soybean, mung bean and alfalfa (Mao 2011; Shuyan 2010), suggesting that *A. lucorum* induces a defense response in the host plant. an increasing number of studies have revealed changes in the transcriptome and metabolome of plants in response to cell-content feeding insects such as mites and thrips (Chen et al. 2020; Huang et al. 2021; Martel et al. 2015; Sarde et al. 2019; Zhang et al. 2020; Huang et al. 2021), salicylic acid was highly enriched only in DEGs with strong induction by *A. lucorum* in cotton (Chen et al. 2020).

In a previous investigation, we found that the degree of damage caused by *A. lucorum* to different varieties of jujube was different, which indicated that different jujube varieties had different resistances to *A. lucorum*. Nonetheless, little is known about the molecular mechanisms of jujube plant defense, especially about defense against sap-feeding mirids such as *A. lucorum*. In the current study, the choice preference of *A. lucorum* for jujubes with different levels of resistance was tested. Transcriptomic changes after infestation by *A. lucorum* of jujube plants with different levels of resistance were analyzed to identify candidate genes and key pathways associated with the activation of jujube plant defense. We also determined the content of signaling compounds (SA and JA) and the expression levels of genes in signaling pathways in jujube leaves infested with *A. lucorum*. Finally, the preference of *A. lucorum* for jujube plants with different levels of resistance after a 36 h exposure to exogenous SA and MeJA was examined. Our results provide a better understanding of the jujube plant response to *A. lucorum* feeding and the mechanisms of induced plant defense against sap-feeding mirids.

Materials And Methods

Plant and insect material

Annual seedlings of Chinese winter jujube (*Ziziphus jujuba* cv. Dongzao) and wild jujube (*Ziziphus jujuba* var. *spinose*) were planted singly in plastic pots (diameter =35 cm, height =30 cm). The seedlings were all grown in a climate chamber at 28 °C on a 16 h light/8 h dark photoperiod.

The nymphs of *A. lucorum* were collected from jujube fields at the National Jujube Germplasm Repository of Shanxi Academy of Agricultural Sciences and subsequently reared in the laboratory following methods described by Lu et al. (2007). The colony was kept in 20 × 10 × 6 cm rearing containers and supplied with green beans (*Phaseolus vulgaris* L.) and a cotton ball soaked with 10% sucrose solution as food (Lu et al. 2007). Newly enclosed adults were used for the experiments.

Plant treatments

Jujube seedlings germinated for one month were used for treatments. Twenty 3-day-old adults of *A. lucorum* were placed on one jujube plant in a nylon mesh cage (length × width × height =60 cm×60 cm×100 cm) starting at 18:00. Jujube plants without *A. lucorum* were treated the same way and used as controls. After 7 d, the bugs were removed and the jujube leaves were sampled by quick freezing in liquid nitrogen and stored at -80 °C for later use.

Behavioral responses of *A. lucorum* to jujube leaves preinfested by *A. lucorum*

Behavioral responses of *A. lucorum* adults were analyzed using a Y-tube olfactometer (Dicke et al. 1990; Pan et al. 2015b). The olfactometer consisted of a 3 cm diameter clear glass tube, with a 30 cm long central tube and two 30 cm lateral arms with a 60° angle. The apparatus was placed in a 100 ×60 × 60 cm dark chamber, illuminated with two 40 W fluorescent lamps (light intensity 2000 lx), and maintained at 28 ± 1 °C and 60 ± 5% RH. A vacuum pressure pump (Beijing Institute of Labor Instrument, Beijing, China) pushed air through activated charcoal and an Erlenmeyer flask filled with distilled water. Airflow through each of the olfactometer arms was maintained at 400 ml/min and entered the apparatus via Teflon tubing (Pan et al. 2021).

A total of 10 different combinations were set in pairs with nothing (blank control), uninfested *Z. jujuba* cv. Dongzao branch, uninfested *Z. jujuba* var. *spinose* branch, *Z. jujuba* cv. Dongzao branch and *Z. jujuba* var. *spinose* branch infested by *A. lucorum*.

Before the behavioral bioassay, *A. lucorum* adults were starved for 6 h. Individual insects were introduced at the base of the main arm of the olfactometer via a 10 cm glass vial. When the *A. lucorum* was one half past the main arm, the timing was started and each insect was given 5 min to respond. A choice of the left or right arm of the olfactometer was noted when the insect moved 3 cm past the Y-junction and stayed there for a minimum of 5 s. If an insect did not make a choice within 5 min, it was removed and recorded as “no choice” (Pan et al. 2021). Each insect was tested only once. The plant tissue was replaced every hour, and the Y-tube was replaced with a clean one after four individuals had been tested. For each treatment, we tested 60 insects of each sex. All bioassays were conducted between 08:00 and 18:00.

RNA extraction and sequencing

RNA was extracted using an RNAprep Pure Polysaccharide Polyphenol Plant Total RNA Extraction Kit (DP441) (Tiagen, Beijing, China). Total RNA was accurately quantified by a Qubit 2.0 RNA detection kit to determine the amount of total RNA used for the library construction. The cDNA library was prepared as previously described (Wang et al. 2009).

The cDNA library was sequenced on the Illumina HiSeq™ 2000 platform, and 100 bp raw PE reads were generated. Raw reads were filtered to obtain clean reads by removing the adaptor sequences, the low-quality sequences, and the reads with unknown base pairs 'N'. All high-quality reads of each sample were aligned to the Musa genome sequence (<http://banana-genome.cirad.fr> version 1) using TopHat 2.0 and Bowtie 2 with default parameters (Trapnell et al., 2009). Cufflinks 1.0.3 (Trapnell et al., 2010) was then used to assemble the transcripts from the TopHat alignment results to determine the gene expression values. Two biological replicates of each sample were used for differential gene expression analysis, and Pearson's correlation coefficients between biological replicates for each sample were calculated in R (logiciel). The genes with absolute values of $\log_2$ (fold change) ≥ 1 and adjusted false discovery rate in q value ≥ 0.99 were identified as differentially expressed genes (DEGs). The gene ontology (GO) functional classification of DEGs (Fisher, P value < 0.05) was carried out by the BLAST2GO 2.5 program (Conesa et al., 2005). Kyoto Encyclopedia of Genes and Genomics (KEGG) enrichment analysis (P value < 0.05) of DEGs was performed using KOBAS2. The gene expression patterns were analyzed with MeV software (www.tm4.org/mev.html) to construct heatmaps.

Real-time RT-PCR for validation of the transcript levels

Gene-specific primers were designed with Primer Premier 5.0 software (Premier Biosoft Interpairs, Palo Alto, CA), and the primer sequences are listed in Table S1. Total RNA was isolated from the xylem of the jujube roots as described for the transcriptome sequencing. First-strand cDNA was synthesized from 1.0 μg of total RNA using the PrimeScript™ RT Master Mix Kit (TaKaRa, Bio Inc., Japan). The rps2 gene was used as the reference gene for normalization (Chen et al., 2011). qRT-PCR was carried out using the SYBR Premix ExTaq Kit (TaKaRa, Bio Inc., Japan) with a reaction (20 μl) including 20 ng cDNA, 0.3 μM of each primer, and 1 $\times$ SYBR Premix ExTaq. All qRT-PCRs were performed on a Light Cycler 480 (Roche Diagnostics, Germany) under the following conditions: 95 °C for 30 s, 40 cycles of 95 °C for 10 s, 60 °C for 20 s and 72 °C for 10 s to calculate the cycle threshold (Ct) values. Each treatment was examined in three technical replicates, and the melt curves were analyzed to ensure primer specificity. The relative expression of each gene was estimated by using the $2^{-\Delta\Delta\text{Ct}}$ method (Livak and Schmittgen, 2001).

Determination of jasmonic acid and salicylic acid

Extraction and analysis of plant hormones were performed according to the method described by Chen et al. (2014).

Behavioral responses of *A. lucorum* to jujube leaves treated with SA and JA

SA (Sigma, USA) and methyl jasmonic acid (MeJA) (Sigma, USA) were both dissolved in ethanol at 2 mM, 1 mM and 0.5 mM. Jujube seedlings that germinated were sprayed with SA (2 mM, 1 mM and 0.5 mM) or MeJA (2 mM, 1 mM and 0.5 mM) solutions and collected 36 h post-spray. Behavioral responses of the *A. lucorum* adults were analyzed using the Y-tube olfactometer as described above. A total of 6 different combinations were set in pairs with CK (sprayed with water (including ethanol)) and treatments (sprayed with SA or MeJA).

Statistical analyses

The relative abundance of the volatile compounds between the herbivore-damaged and corresponding cotton controls were compared, and the data were analyzed for normality before performing Student's *t*-tests. For the Y-tube olfactometer assays, *A. lucorum* preferences were analyzed using χ^2 goodness-of-fit tests with the null hypothesis that *A. lucorum* had no preference for either olfactometer arm (i.e., a 50:50 response). Data analysis was performed with SAS 9.4 software (SAS Institute 2012).

Results

Analysis of jujube resistance to *A. lucorum*

Winter jujube was one of the most sensitive varieties to *A. lucorum*. Wild jujube showed more resistance and nonselectivity to *A. lucorum* (Fig. 1). In the field, the number of winter jujube and wild jujube leaves did not change significantly after *A. lucorum* feeding (Fig. S1). However, there were many holes in the new leaves fed on by *A. lucorum*. Compared with the control, the leaf area of winter jujube decreased by 78.05%, and the leaf area of wild jujube leaves was 34.18% less than that of control leaves (Fig. S2).

A. lucorum significantly tended to select healthy jujube leaves over the blank control. Comparing the uninjured resistant varieties (wild jujube) and the uninjured susceptible varieties (winter jujube), 75.00% of the *A. lucorum* tended to select winter jujube, and only 20% chose wild jujube. There was no difference in the choice between damaged wild jujube and the blank control. Between the damaged winter jujube and the blank control, 58.30% of *A. lucorum* chose the leaves of winter jujube, and 28.33% chose the blank. Between damaged and undamaged leaves of the same type, 63.33% of the *A. lucorum* tended to choose the undamaged winter jujube leaves and 75.00% significantly tended to select undamaged wild jujube leaves (Fig. 2).

Differentially expressed genes (DEGs) between winter jujube and wild jujube

The numbers of DEGs between pairs of samples were determined (Fig. 3). When comparing samples from *A. lucorum* infestation with those of the control treatment, there were 438 genes that had significant changes, among which 274 genes were upregulated and 164 genes were downregulated. After wild jujube was infested, 796 genes had significant changes, of which 464 genes were upregulated and 332 genes were downregulated. After damage by *A. lucorum*, 2339 genes in the leaves of winter jujube were changed compared with those of wild jujube leaves. Among them, 1222 genes were upregulated, and

1117 genes were downregulated. Compared with healthy wild jujube leaves, 2327 genes in unharmed winter jujube leaves changed, of which 1109 genes were upregulated and 1218 genes were downregulated. Venn diagrams were created to analyze the similarities and differences between winter jujube and wild jujube before and after infestation by *A. lucorum*. There were 95 DEGs in the different treatments, but more DEGs were exclusive to either winter jujube or wild jujube (Fig. 3).

KEGG pathway functional enrichment and GO enrichment analysis of the DEGs

KEGG enrichment analysis was performed of the two groups (DT vs. DCK, ST vs. SCK). A total of 93 DEGs between DT and DCK were mapped to 85 KEGG pathways, including several pathways related to the defense response against pests (Fig. 4). Fourteen DEGs were enriched in the term plant hormone signal transduction (ko04075, 14/273), which was the most highly represented pathway. Seven DEGs were enriched for the term phenylpropanoid biosynthesis (ko00940, 7/234), and 7 other DEGs were enriched for the term plant–pathogen interactions (ko04626, 7/293).

A total of 172 DEGs between ST and SCK were mapped to 149 KEGG pathways, including several pathways related to the defense response against pests (Fig. 5). Fifteen DEGs were enriched in the term plant–pathogen interactions (ko04626, 15/293), which was the most highly represented pathway. Eleven DEGs were enriched for the term plant hormone signal transduction (ko04075, 11/273). Fourteen DEGs were enriched for the term biosynthesis of amino acids (ko01230, 14/281), and 14 other DEGs were enriched for biosynthesis of amino acids (ko01200, 13/269).

To further investigate the biological functions of the DEGs and verify the results of the KEGG analysis, the GO classifications of DEGs in the DT vs. DCK and ST vs. SCK comparisons were compared. The DEGs (T/CK) of the two groups (DT vs. DCK, ST vs. SCK) were classified into three categories: 'biological processes', 'cellular components' and 'molecular functions' (Fig. 5, Fig. 6). Similar to the results of the KEGG pathway enrichment analysis, "response to chemical", "response to hormone", "hormone-mediated signaling pathway", "cellular response to hormone stimulus" and "cellular response to endogenous stimulus" were represented, and the number of DEGs was significantly higher in DT vs. DCK (Fig. 5). "Response to stimulus", "response to stress", "response to chemical", and "response to biotic stimulus" were represented, and the number of DEGs was significantly increased in SZT vs. SZCK (Fig. 6).

In addition, "response to jasmonic acid" (13 genes), "jasmonic acid mediated signaling pathway" (8 genes), "cellular response to jasmonic acid stimulus" (8 genes), "regulation of jasmonic acid mediated signaling pathway" (2 genes), "induced systemic resistance" (1 gene) and "response to salicylic acid" (10 genes) were enriched in the DT vs. DCK comparison (Fig. 9). After *A. lucorum* infestation, 9 of 13 genes in the "response to jasmonic acid" pathway of winter jujube were upregulated, and 4 genes were downregulated, while 6 of 10 genes in the "response to salicylic acid" pathway were downregulated, and 4 genes were upregulated.

Meanwhile, "response to jasmonic acid" (16 genes), "jasmonic acid mediated signaling pathway" (6 genes), "cellular response to jasmonic acid stimulus" (6 genes), "induced systemic resistance, jasmonic

acid mediated signaling pathway" (2 genes), "response to salicylic acid" (9 genes) and "salicylic acid mediated signaling pathway" (2 genes) were also "enriched in the SZT vs. SZCK comparison. After *A. lucorum* infestation, 12 of 16 genes in the "response to jasmonic acid" pathway of wild jujube were upregulated, and 4 genes were downregulated, while 7 of 9 genes in the "response to salicylic acid" pathway were downregulated, and 2 genes were upregulated. This suggests that plant hormone signal transduction plays a key role in the defense response of jujube to *A. lucorum* (Fig. 8).

Determination of the content of signal substances in the leaves of different jujube varieties

The feeding of *A. lucorum* induced an increase in jasmonic acid content in jujube leaves while inhibiting the accumulation of salicylic acid (Fig. 10). The content of jasmonic acid in winter jujube leaves after *A. lucorum* feeding was increased by 32.79% compared to the control, while the content of jasmonic acid in wild jujube leaves was increased by 51.89% (Fig. 10a). The salicylic acid content in the leaves of winter jujube decreased by 73.76% after *A. lucorum* damage, while it decreased by 44.09% in the leaves of wild jujube (Fig. 10b). The contents of jasmonic acid and salicylic acid in the healthy leaves of winter jujube were significantly lower than those of wild jujube leaves.

Field investigations found that the damage caused by *A. lucorum* to wild jujube leaves was significantly less than that of winter jujube leaves. The selection experiment also showed that the selection rate of *A. lucorum* for winter jujube leaves was significantly higher than that for wild jujube leaves (Fig. 2). This finding indicates that the SA and JA signaling pathways may play an important role in the basic defense of jujube against *A. lucorum*.

Expression of key genes of defense signaling pathways in leaves of different jujube varieties

Isochorismate synthase (ICS) and phenylalanine ammonia lyase (*PAL*) are required to synthesize salicylic acid for plant defense (Filgueiras et al. 2019; Lefevere et al. 2020; Shine et al. 2016; Wildermuth et al. 2001). After *A. lucorum* feeding, the expression of the *PAL* and *ICS2* genes in both winter jujube and wild jujube was significantly downregulated (Fig. 11a, b). In winter jujube leaves, *PAL* and *ICS2* decreased by 20.53% and 32.09%, respectively, while *PAL* and *ICS2* of wild jujube decreased by 18.09% and 24.41% (Fig. 11a, b). In healthy plants, the *PAL* gene expression level in wild jujube leaves was not significantly different from that of winter jujube leaves (Fig. 1b), while the expression level of the *ICS2* gene of wild jujube leaves was higher than that of winter jujube leaves (Fig. 11a).

Nonexpresser of PR genes 1 (*NPR1*) plays a key role in connecting SA and downstream responses (Ding et al. 2018; Peng et al. 2021). The expression of *NPR1* was significantly downregulated in winter jujube leaves after *A. lucorum* infestation but did not change in wild jujube leaves (Fig. 11c). The Phytoalexin deficient4 gene (*PAD4*) is important in the regulation of the plant defense pathway involving SA signaling (Cui et al. 2017; Wiermer et al. 2005). The expression levels of *PAD4* in winter jujube and wild jujube leaves both decreased after *A. lucorum* feeding (Fig. 11d). Plant PR proteins are involved in various types of pathogen infections, such as infections with viruses, bacteria, and fungi (Ali et al. 2018). PR genes are SA signature genes that induce [systemic acquired resistance](#) (SAR) (Ali et al. 2018; Ali et al. 2017). Plant

β -1,3-glucanases (BGL2) belong to the PR-2 family and reportedly play an important role in plant defense responses (Xie et al. 2015). The PR1-1, BGL2 and PR1-2 genes were significantly downregulated after *A. lucorum* harmed winter jujube and wild jujube leaves (Fig. 11). In the winter jujube CK plants, the expression levels of NPR1, PR1-1, PR1-2 and BGL2 were higher than those of wild jujube leaves (Fig. 11).

It is well known that the biosynthesis of JA mainly starts with the release of α -linolenic acid (α -LeA) from galactolipids of the chloroplast membranes (Wang et al. 2018; Wasternack and Feussner 2018). The substrate α -LeA is catalyzed into cis-(+)-12-oxo-phytodienoic acid (OPDA) under the sequential action of a series of chloroplast-located enzymes, including 13-lipoxygenase (13-LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) (Wang et al. 2019; Wasternack and Hause 2013). After damage by *A. lucorum*, the expression levels of LOX3, AOS, AOC3 and AOC4 in winter jujube leaves increased by 25.88%, 24.89%, 22.92% and 37.51% compared to the undamaged leaves, while they increased by 22.77%, 38.77%, 7.34% and 10.42% relative to damaged wild jujube (Fig. 12). In healthy plants, the gene expression levels of LOX3 and AOS in wild jujube leaves were 39.52% and 55.33% higher than those in winter jujube leaves. After damage by *A. lucorum*, the expression of LOX3 and AOS genes in the wild jujube increased by 36.08% and 72.53%, respectively, compared with that in winter jujube (Fig. 12). After *A. lucorum* infestation, the expression of the AOC3 and AOC4 genes in wild jujube leaves was reduced by 20.44% and 29.73%, respectively, compared with that in winter jujube leaves (Fig. 12).

The perception of insect attack locally and systemically elicits rapid synthesis of jasmonate, which is perceived by the F-box protein COI1 to further recruit JAZ repressors for ubiquitination and degradation, thereby releasing transcription factors that subsequently activate the plant defense against insect attack (Wang et al. 2019). Expression of the COI gene of winter jujube and wild jujube increased by 16.86% and 36.89%, respectively, after damage by *A. lucorum* (Fig. 12e). COI expression in the healthy leaves of wild jujube was 19.22% higher than that of the healthy leaves of winter jujube. COI expression in the leaves of wild jujube after damage by *A. lucorum* was 39.69% higher than that of winter jujube (Fig. 12e). FAD3 was significantly upregulated in winter jujube and wild jujube leaves after *A. lucorum* feeding, but the relative expression levels of FAD3 in the wild jujube plants were 97.29% higher than those in winter jujube leaves after *A. lucorum* damage (Fig. 12f).

Effect of SA and MeJA on the feeding selection behavior of *A. lucorum*

There was no significant difference in the feeding selection rate of *A. lucorum* between jujube leaves sprayed with 0.5 mM SA and those sprayed with water (Fig. 13). After spraying 1 mM SA on wild jujube and winter jujube, the selection rates of *A. lucorum* were 42.44% and 45.00%, respectively, while the selection rates of *A. lucorum* after spraying them with water were 56.11% and 52.78%, respectively. Between 2 mM SA-sprayed and the water-sprayed wild jujube, 61.11% and 55.83% of *A. lucorum* significantly tended to prefer water-sprayed wild jujube and winter jujube leaves, respectively (Fig. 13). After SA treatment, the repellent effect of wild jujube leaves was stronger than that of winter jujube leaves. Moreover, the higher the concentration of salicylic acid, the more obvious the repellent effect of the jujube leaves.

After the jujube leaves were sprayed with jasmonic acid, they had an obvious repelling effect on *A. lucorum*, and the repelling effect became stronger as the spraying concentration increased. After spraying 0.5 mM, 1 mM and 2 mM jasmonic acid on wild jujube leaves, the proportions of *A. lucorum* selected for water spray treatment were 58.33%, 61.67% and 72.22%, respectively (Fig. 13).

After spraying the leaves of winter jujube with 0.5 mM, 1 mM and 2 mM jasmonic acid, the proportions of *A. lucorum* selected for water spray treatment were 50.11%, 60.22% and 65.22%, respectively (Fig. 13). When spraying 2 mM JA, the avoidance effect of wild jujube and winter jujube leaves was the strongest. After the application of jasmonic acid, the avoidance effect of wild jujube leaves was significantly stronger than that of winter jujube leaves.

Discussion

All crop plants have some chemical or physical characteristics that reduce their suitability for potential pests as a result of both natural selection and human selection by generations of farmers (Stoner 1996). Historically, plant breeders have developed resistance to insects in situations where it was perceived to provide economic advantages over the use of insecticides in conventional agriculture (Hill et al. 2004). Under these circumstances, the use of resistant varieties is the principal method of control for many key insect pests (Walling 2000). There are significant differences in the resistance levels of different crop varieties to *A. lucorum*. For example, Wang et al. (2017) reported that 13 grape varieties, including 'Xiangyue', 'Merlot', and 'Beibinghong', are highly resistant to *A. lucorum*; among them, 'Xiangyue' has the strongest resistance to *A. lucorum*. Eleven varieties, including Jintian Meizhi, Jiafei Yunu, and Jinfinger, are highly susceptible, among which Jintian Meizhi has the lowest resistance index. Luo et al. (2011) selected 5 strains resistant to *A. lucorum* and 1 strain that was susceptible to *A. lucorum* from among more than 210 cotton varieties. In the field, winter jujube was found to be a susceptible strain, while wild jujube was a resistant strain.

Plant resistance can be divided into 3 forms, namely, antixenosis, defensiveness and tolerance (Smith and Clement 2012). Antixenosis is a type of resistance in which a plant is relatively less exploited by an insect for feeding, oviposition or shelter (de Queiroz et al. 2020; Stec et al. 2021). This resistance results from plant morphology, chemical constituents and physical characteristics (de Queiroz et al. 2020; Seifi et al. 2013). The antixenosis type of resistance can be mediated by plant visual, olfactory and/or gustatory cues (Diego et al. 2015; Sanches et al. 2017). When the winter jujube and wild jujube coexisted, *A. lucorum* showed obvious positive tropism to Dongzao. The results showed that wild jujube has a strong antixenosis to *A. lucorum* (Fig. 2). After *A. lucorum* infestation, the healthy leaf area of Dongzao jujube decreased significantly compared with that of wild jujube (Fig. 1; Fig. S2), which indicated that the wild jujube has a strong defensive effect against *A. lucorum*.

Rapid and low-cost high-throughput deep sequencing technology has been successfully used for comparative genomics, expression profiling and molecular mechanism investigation of plants after pest infestation (Ali et al. 2017; Dubey et al. 2013). The number of upregulated genes was significantly higher

than that of downregulated genes after *A. lucorum* fed on winter jujube (susceptible variety) and wild jujube (resistant variety) (Fig. 3). This was consistent with transcriptomic changes in cotton plants responding to *A. lucorum* (Huang et al. (2021)). These results were similar to the effects of various chewing insects on the transcriptome of host plants, such as cotton bollworm (Huang et al. 2015), cotton boll weevil (Artico et al. 2014), *Chilo suppressalis* (Liu et al. 2016), and *Ostrinia furnacalis* (Guo et al. 2019).

A further distinction can be made between both constitutive and inducible plant defenses, and each can be either direct or indirect (Mithofer and Boland 2012). The defense responses induced by herbivores with different feeding behaviors were different (Huang et al. 2021). Different phytohormones, such as JA, ET and SA, mediate defenses against insect herbivore attack (Erb and Reymond 2019). Distinct transcriptional responses and signal transduction pathways are often activated in plants in response to herbivores depending on the attacker and its feeding type (Stam et al. 2014; Erb and Reymond 2019). For example, chewing herbivores commonly induce a defense response mediated by the JA pathway (STEPPUHN et al. 2008). However, phloem feeders usually induce the SA pathway (Donovan et al. 2013; Zarate et al. 2007). Because mirids (order: Hemiptera, suborder: Heteroptera, family: Miridae) typically employ “lacerate and flush” feeding strategies to cut plant cells (Showmaker et al. 2016), they cause more physical damage to cells than phloem-feeding aphids and whiteflies. The transcriptomic results indicated that *A. lucorum* infestation upregulated genes of the JA and SA pathways in cotton (Chen et al. 2020; Huang et al. 2021). With KEGG pathway functional enrichment and GO enrichment analysis of the DEGs, *A. lucorum* infestation changed the expression of genes in the JA and SA pathways in winter jujube and wild jujube (Fig. 8 and Fig. 9).

There is antagonism between the salicylic acid pathway and jasmonic acid pathway (Mur et al. 2006). Analysis of the hormones revealed enhanced levels of SA, while JA levels were strongly diminished when maize was infested by *Colletotrichum graminicola* (Gorman et al. 2020). The silverleaf whitefly induces salicylic acid defenses and suppresses effective jasmonic acid defenses (Zarate et al. 2007). However, chewing caterpillars may induce JA-responsive genes that influence the activity of primary SA-responsive genes activated by pathogens and sap-sucking whiteflies or aphids (Kessler and Baldwin 2002). In cotton plants, *A. lucorum* infestation induced the JA pathway without attenuating the SA pathway (Huang et al. 2021). The JA and SA pathways of cotton acted synergistically after *A. lucorum* infestation (Huang et al. 2021). Additionally, SA signaling plays a more important role in defense against *A. lucorum* in cotton (Chen et al. 2020). According to the GO classifications, *A. lucorum* infestation upregulated the JA pathway and downregulated the SA pathway in jujube leaves (Fig. 8. 9). Our data showed that in jujube leaves, *A. lucorum* induced an increase in the level of jasmonic acid and inhibited the accumulation of salicylic acid (Fig. 10).

JA, derived from α -linolenic acid via one branch of the octadecanoid pathway, is an important regulator of defense responses against many herbivores. We observed the activation of many genes involved in JA biosynthesis and signaling pathways, such as LOX3, AOS, AOC3, AOC4 COI and FAD3. These findings are

similar to those reported for different plant responses to chewing pests (Huang et al. 2015; Zhou et al. 2011). This indicates that after *A. lucorum* infestation, JA signal transduction may act through a mechanism in jujube similar to that in other plants fed on by chewing insects. Meanwhile, we also found that in contrast to the high induction of jasmonate-related genes, some genes related to SA biosynthesis were downregulated, including PAL and ICS2, the crucial enzymes that contribute to SA biosynthesis; NPR1, PAD4, and BGL2; and two genes that produce the SA signaling protein PR1. It is possible that the JA signaling pathway elicited by *A. lucorum* may suppress SA-related genes. This is consistent with antagonistic effects in cotton during an infestation of the cotton bollworm *Helicoverpa armigera* (Huang et al. 2015) or in *Arabidopsis thaliana* infested by *Spodoptera exigua* (Cipollini et al. 2004).

Arabidopsis mutants of JA biosynthesis genes (such as *aos* and *opr3*) or the receptor gene (*coi1*) are significantly more susceptible to insects than wild-type plants (Browse 2009; Fonseca et al. 2009), while the higher-order mutants *jazQ* and *jazD* exhibit stronger resistance to herbivores (Campos et al. 2016; Guo et al. 2018; Major et al. 2017). Silencing OsPALs also resulted in reduced SA levels and compromised resistance to brown plant hoppers, suggesting that SA produced from the PAL pathway contributes to insect resistance in rice (He et al. 2020). The contents of jasmonic acid and salicylic acid in the healthy leaves of winter jujube were significantly lower than those in wild jujube leaves (Fig. 10). Compared with winter jujube, wild jujube showed stronger resistance (Fig. 1). These results showed that salicylic acid and jasmonic acid might play an important role in basic defense against *A. lucorum* in jujube leaves.

JA, cis-jasmone (CJ) and MeJA are known to have repellent, deterrent and/or antixenotic effects on aphid species attacking cereals and cotton (Bruce et al. 2003; El-Wakeil et al. 2010; Omer et al. 2001). MeJA treatments have repellent effects on aphids, phytophagous thrips and hoverflies (Bayram 2018). The preference of *F. occidentalis* was restricted by JA application on *Arabidopsis* and *Brassica rapa* plants (Abe et al. 2009). Methyl salicylate (MeSa) is a plant-produced volatile derivative of salicylic acid (Shulaev et al. 1997). MeSA is repellent against an array of harmful insect species, including the yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae) (Dekker et al. 2011), redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae) (Hughes et al. 2017), and black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae) (Hardie et al. 1994). A low concentration (0.5 mM) of SA on jujube leaves had no repellent effect on *A. lucorum*, but a high concentration (2 mM) of SA restricted the preference of *A. lucorum*. Exogenous JA had an obvious repelling effect on *A. lucorum* in jujube leaves. After exogenous SA and JA treatment, the repellent effect of wild jujube leaves was stronger than that of winter jujube leaves. Moreover, the higher the concentrations of exogenous SA and JA were, the more obvious the repellent effect on *A. lucorum* (Fig. 12). After *A. lucorum* feeding, the jujube leaves showed strong repellency to *A. lucorum* compared with healthy leaves (Fig. 2). Combining the SA and JA concentration changes (Fig. 12), we found that JA in jujube leaves played an important role in the defense against *A. lucorum*.

Declarations

Author contributions

QL and WT conceived and designed the experimental plan. QL WT, XS, HC, DY, and ZJ performed the experiments. WT, XS and ZJ refined the manuscript. All authors approved the final manuscript.

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Figures



Figure 1

Characters of winter jujube and wild jujube leaves infested by *Apolygus lucorum*. *a* Winter jujube infested by *A. lucorum*. *b* Wild jujube infested by *A. lucorum*.

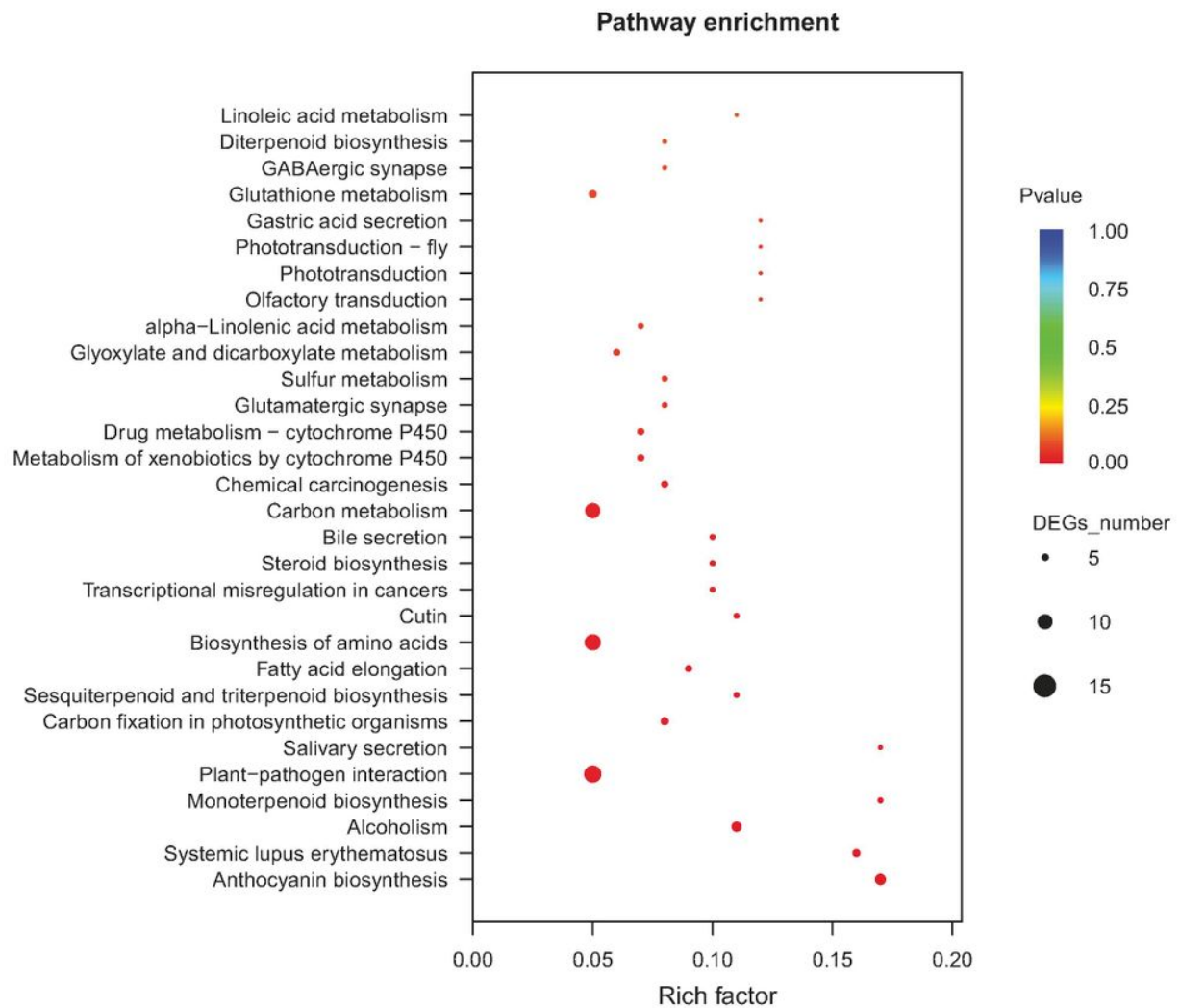


Figure 5

Pathway enrichment for Wild jujube-CK vs Wild jujube-T. Wild jujube-CK was the health wild jujube leaves. Wild jujube-T was wild jujube leaves infested by *A. lucorum*. The y-axis represents KEGG pathways; the x-axis represents enrichment factors; larger values indicate a higher percentage of the DEGs in the pathway.

Supplementary Files

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