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Transcriptomic analysis of melon with different *Phelipanche aegyptiaca* resistance

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Abstract: To elucidate the genetic factors contributing to melon resistance against *Phelipanche aegyptiaca* and comprehend the role of differentially resistant materials in responding to changes in *P. aegyptiaca* parasitisation, we investigated the *P. aegyptiaca*-resistant line K16 and the susceptible line K27. The parasitism rate of *P. aegyptiaca* was assessed at 25 days. Results revealed significant differences in parasitisation rates between K16 (15.35%) and K27 (34.2%). We compared inoculated K16 and K27 to their respective controls through transcriptome analysis and contrasted inoculated K16 with inoculated K27. Eight hundred eighteen genes exhibited differential expression across all comparisons. Gene ontology (GO) functional enrichment analysis revealed that differentially expressed genes were significantly enriched in nitrate transport and assimilation, cellular components, extracellular regions, binding and enzyme activities. KEGG pathway enrichment underscored the importance of phytohormone signaling, phenylpropanoid biosynthesis, linolenic acid and linoleic acid metabolism, cyanoamino acid metabolism and nitrogen metabolism in the interaction between melon and *P. aegyptiaca*. Nine genes potentially associated with *P. aegyptiaca* resistance were identified, encoding cytochrome protein P450, peroxidases, β-glucosidase, acyltransferase family proteins, histidine phosphotransfer protein, and D-type cyclins. This study aims to provide insights into the mechanism of *P. aegyptiaca* parasitism on melons and offers implications for breeding resistant varieties.

Keywords: melon; *Phelipanche aegyptiaca (*Pers.); transcriptome; differentially expressed genes

Melon (*Cucumis melo* L.) is a pivotal cash crop within the Cucurbitaceae family, globally recognised for its nutritional richness and high market demand. With an extensive history of cultivation, Xinjiang emerges as the foremost melon-producing area in China. Over decades of continuous development, the melon industry has evolved into a cornerstone of Xinjiang's agriculture, pivotal in the region's "rural revitalisation" strategy and achieving local financial independence. Despite these advancements, Xinjiang faces a critical challenge as it contends with the pervasive presence of *Phelipanche aegyptiaca*, which has established itself as the most widely distributed and severe

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threat to melon cultivation in China. The rapid proliferation of *P. aegyptiaca* in both southern and northern melon production areas of Xinjiang poses a substantial menace, resulting in reduced yields of 3 500–5 000 hm^2 per year and the potential extinction of melon crops over 1 500–2 000 hm^2 (Zhang et al. 2012; Bai 2020; Cao et al. 2020). Notably, *P. aegyptiaca* has been identified as a particularly dangerous quarantine pest in Xinjiang. Effectively addressing the profound damage caused by melon lechon has thus become an imperative challenge that must be overcome to ensure the sustainable development of melon production in Xinjiang.

P. aegyptiaca, a pernicious root parasitic seed plant belonging to the *Orobanche* in the family *Orobanchaceae*, encompasses over 200 species. Among these, *P. aegyptiaca* is known to infest melons, causing substantial damage (Yao 2016; Peng 2018; Zhang et al. 2021a). Once melons are susceptible to this parasitic plant, normal growth and development become challenging, leading to symptoms such as dwarfing, wilting, dying and other symptoms. This can lead to a reduction in biomass and a complete lack of production harvest. Additionally, *P. aegyptiaca* parasitism significantly impacts fruit quality, diminishing the market value of the produce (Longo et al. 2010). The characteristics of *P. aegyptiaca*, including prolific seed production (104 pcs/plant), a broad parasitism range, severe damage potential, multiple transmission routes, and a long survival time (15–20 years), contribute to its rapid spread, making prevention and control challenging (Yaacoby et al. 2015; Peng 2018). Traditional agricultural control measures are costly, time-consuming and labour-intensive, offering limited eradication possibilities. Chemical control methods only target the aboveground part of the *P. aegyptiaca*, proving ineffective against seed germination and parasitism and pose security risks with potential adverse effects on crop health and environmental pollution. Biological control efforts are primarily in the research and experimental stages, with limited reports on field extension and large-scale applications (Rubiales & Fernández-Aparicio 2012; Dong et al. 2016; Bai 2020). Therefore, it is particularly important to cultivate resistant varieties that represent the most economical, effective, and environmentally friendly approach to melon control. However, research on breeding melons for *P. aegyptiaca* resistance lags, with no identified resistance genes and an absence of understanding regarding resistance mechanisms.

Building upon the current challenges outlined, this study utilised the pre-identified melon materials characterised by resistance and susceptibility to *P. aegyptiaca*. Through transcriptome analysis, we scrutinised the alterations in key genes within the resistance-differentiated materials in response to *P. aegyptiaca* infestation. The objective is to furnish a genetic foundation for future investigations to breed melon varieties resilient to *P. aegyptiaca*.

MATERIAL AND METHODS

Test material. The Hami Melon Research Center of Xinjiang Academy of Agricultural Sciences has diligently preserved melon materials, specifically K16, known for its resistance to *P. aegyptiaca*, and K27, identified as susceptible to *P. aegyptiaca*. Additionally, samples of the *P. aegyptiaca* pest have been meticulously maintained by the Center.

Inoculation and sampling. According to the method of Zhang et al. (2019), a single melon seedling was vertically placed in the middle of a polyethylene bag pre-placed with glass fibre filter paper, exposing part or all the cotyledon. Subsequently, 20 mg of *P. aegyptiaca* seeds were evenly distributed into each bag. The polyethylene bag was placed on a culture rack surrounded by the black film (keeping the roots in darkness). A total of 20 mL of Hoagland nutrient solution was added, and the cultures were maintained at a temperature of 25 °C/20 °C (day/night) with a 14 h light cycle. This process was repeated for two melon varieties, each with ten replicates. The parasitism rate was calculated 25 days after the initiation of the experiment. Root samples from the resistant strain K16 (designated as R0 and R25) and the susceptible strain K27 (designated as F0 and F25) were collected at both 0 days (R0 and F0 as controls) and 25 days after inoculation. Three replicates were obtained for each sample. Immediately following collection, the samples were frozen in liquid nitrogen and stored in a refrigerator at −80 °C for subsequent sequencing. The parasitism rate was calculated using the formula:

$$\text{Parasitism rate} = \frac{\text{No. of parasitised melon plants in the community}}{\text{Total No. of melon plants in the community}} \times 100$$

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RNA-seq library preparation and sequencing. The RNA sequencing was outsourced to Nanjing Jisi Huiyuan Biotechnology Co. (Nanjing, China). Eukaryotic mRNA was enriched with magnetic beads with Oligo (dT), and the mRNA was broken into short fragments by adding an interruption reagent. The first cDNA strand was synthesised with six-base random primers (random hexamers) using mRNA as a template, and then the second cDNA strand was synthesised by adding dNTPs, RNase H, and DNA polymerase I, and the cDNA was purified. The double-stranded cDNA, now purified, underwent end repair, A-tailing, and ligation with sequencing junctions. Subsequently, the cDNA library was enriched through PCR after fragment size selection using AMPure XP beads. After the library construction was completed, the quality of the library was examined. Qubit (version 2.0) was used for preliminary quantification, and Agilent (version 2100) was used to detect the insert size of the library. qRT-PCR was used to accurately quantify the effective concentration of the libraries (with a concentration greater than 2 nM). The libraries were checked and passed, and different libraries were assembled according to the target data volume and sequenced using the NovaSeq Control Software (version 1.7) with a sequencing read length of PE150.

Assembly screening, functional annotation and enrichment analysis of differentially expressed genes. Raw data was filtered to obtain high-quality clean reads, which were aligned to the reference genome to obtain mapped data. The quality of sequencing libraries was evaluated through insertion fragment length tests, randomness tests, and other assessments. Additionally, the quality of expression analysis, variable splicing analysis, new gene discovery, and gene structure optimisation were assessed based on mapped data. Expression analysis, variable splicing analysis, new gene mining and gene structure optimisation were performed using the expression levels of genes across different samples or sample groups.

Data processing. Data processing involved transcriptome assembly and quantification using Stringtie (version 2.1.3b). Statistical analysis of bam comparison results was conducted using Qualimap (version 2.2.1). Biological replicates underwent variable shear analysis with rMATS (version 3.1.0). Heatmap plotting was performed using R Studio (version 4.3.2). Replicate differences were analysed using DESeq (version 2 1.26.0), and differences were also examined using edgeR (version 3.28.1).

RESULTS

Evaluation of melon varieties inoculated with *P. aegyptiaca*. To assess the effectiveness of melon resistance to *P. aegyptiaca*, we conducted inoculation experiments, monitoring the parasitisation rates of both the resistant line K16 and the susceptible line K27 after 25 days. Notably, we observed a substantial difference in resistance (Figure 1A), with the *P. aegyptiaca* parasitisation rate of K16 at 15.35%, significantly lower than that of K27 at 34.20% (Figure 1B). During our investigation into the parasitisation rate, a striking observation emerged: the mortality rate of *P. aegyptiaca* in the sensitive line K27 reached 39.56% after parasitisation, a stark contrast to the mere 5.27% observed in the *P. aegyptiaca*-resistant line (Figure 1C). This intriguing finding led us to formulate a hypothesis suggesting that K27 induces the death of parasitised *P. aegyptiaca*, potentially as a mechanism to develop resistance. This hypothesis sets the stage for a deeper exploration into the underlying resistance mechanisms, further justifying the subsequent transcriptome analysis with K16 and K27.

Transcriptome analysis of melon varieties upon *P. aegyptiaca* infestation. To unravel the molecular responses of melon varieties to *P. aegyptiaca* infestation, we performed transcriptome analysis on samples collected at 0 and 25 days post-parasitism. RNA sequencing yielded high-quality data for each sample, with clean reads exceeding 6.77 Gb, guanine(G) and cytosine(C) content not falling below 43.66%, and Q30 bases constituting 90.07% or more (Table 1). The clean reads of each sample were aligned with the melon reference genome (DHL92_genome_v4, http://www.cucurbitgenomics.org/) for sequence comparison, achieving a comparison efficiency surpassing 93.08%. Notably, the calculated Pearson's correlation coefficients between the samples, based on gene expression, consistently exceeded 0.98 for all pairs, indicating high data accuracy. This robust dataset forms a solid foundation for our in-depth exploration of melon responses to *P. aegyptiaca* at the transcriptome level.

Differential gene expression in response to *P. aegyptiaca* infestation. Using RNA sequenc-

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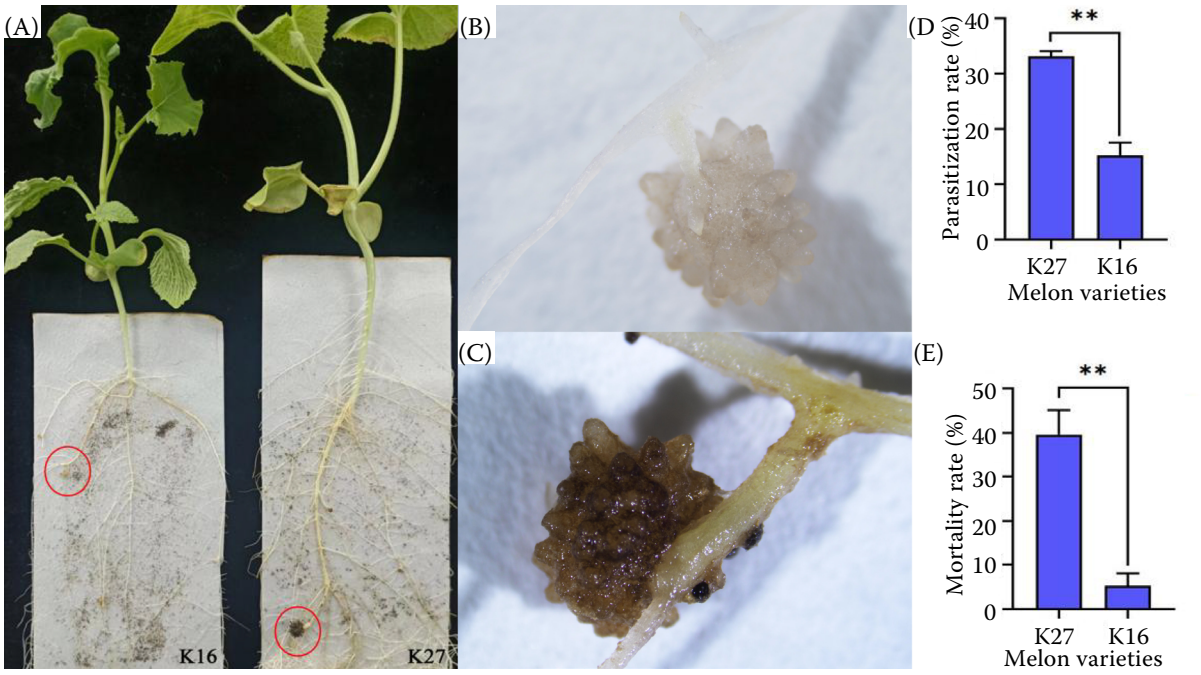

Figure 1. Evaluation of *Phelipanche aegyptiaca* parasitic resistance in melon

Root parasitisation observed in resistant K16 and susceptible K27 lines 25-day post-inoculation with *P. aegyptiaca*; red circles highlight the parasitised parts of *P. aegyptiaca*, with the black *P. aegyptiaca* in the red circle of K27 indicating the mortality of parasites; (B) K16 lines 25-day post-inoculation with *P. aegyptiaca* of *P. aegyptiaca;* (C) K27 lines 25-day post-inoculation with *P. aegyptiaca* of *P. aegyptiaca*; (D) quantification of the *P. aegyptiaca* parasitisation rate; (E) calculation of the mortality rate of parasitised *P. aegyptiaca*; data are presented as mean ± SE (n = 3, t-test, $^{**}P < 0.01$)

ing data, we identified differentially expressed genes (DEGs) in response to *P. aegyptiaca* infestation in the resistant line K16 and the susceptible line K27. After 25 days of *P. aegyptiaca* parasitism, distinctive gene expression patterns emerged in each experimental group. In the susceptible K27 line, *P. aegyptiaca* parasitism led to significant changes, revealing 4 847 DEGs. Among these, 2 457 genes were upregulated, while 2 390 were down-regulated (Figure 2A). Similarly, in the resistant line K16 (R25) compared to its control (R0), 4 578 DEGs were identified, comprising 2 197 upregulated genes and 2 381 down-regulated genes (Figure 2B). Furthermore, when contrasting the susceptible line (F25) with the resistant line (R25), 3 769 DEGs were observed. Of these, 2 141 genes were upregulated, and 1 628 were down-regulated (Figure 2C). Notably, 818 genes exhibited differential expression across all treatments, indicating a shared set of genes responding to *P. aegyptiaca* infestation regardless of melon variety (Figure 2D). These findings offer valuable insights into the nuanced transcriptional responses of melon varieties to *P. aegyptiaca* and set the stage for further functional exploration of these DEGs.

Gene Ontology (GO) functional annotation of DEGs upon *P. aegyptiaca* infestation. To elucidate the functional implications of differentially ex-

Table 1. Quality control and statistics of transcriptome data

Sample	Read sum	Base sum	GC (%)	Q20 (%)	Q30 (%)	Reads aligned
F0	27 043 861.00	8 113 158 300	43.93	96.64	91.01	51 995 506 (96.13%)
F25	27 096 274.67	8 128 882 400	43.71	96.69	90.94	50 565 248 (93.31%)
R0	26 198 950.67	7 859 685 200	44.21	96.23	90.91	50 274 431 (95.95%)
R25	24 500 096.00	7 350 028 800	43.95	96.74	90.44	45 835 158 (93.57%)

GC (%) – the percentage of total bases in clean data that are guanine (G) and cytosine (C) bases; Q20 (%) – percentage of bases with clean data mass values ≥ 20; Q 30(%) – percentage of bases with clean data mass values ≥ 30

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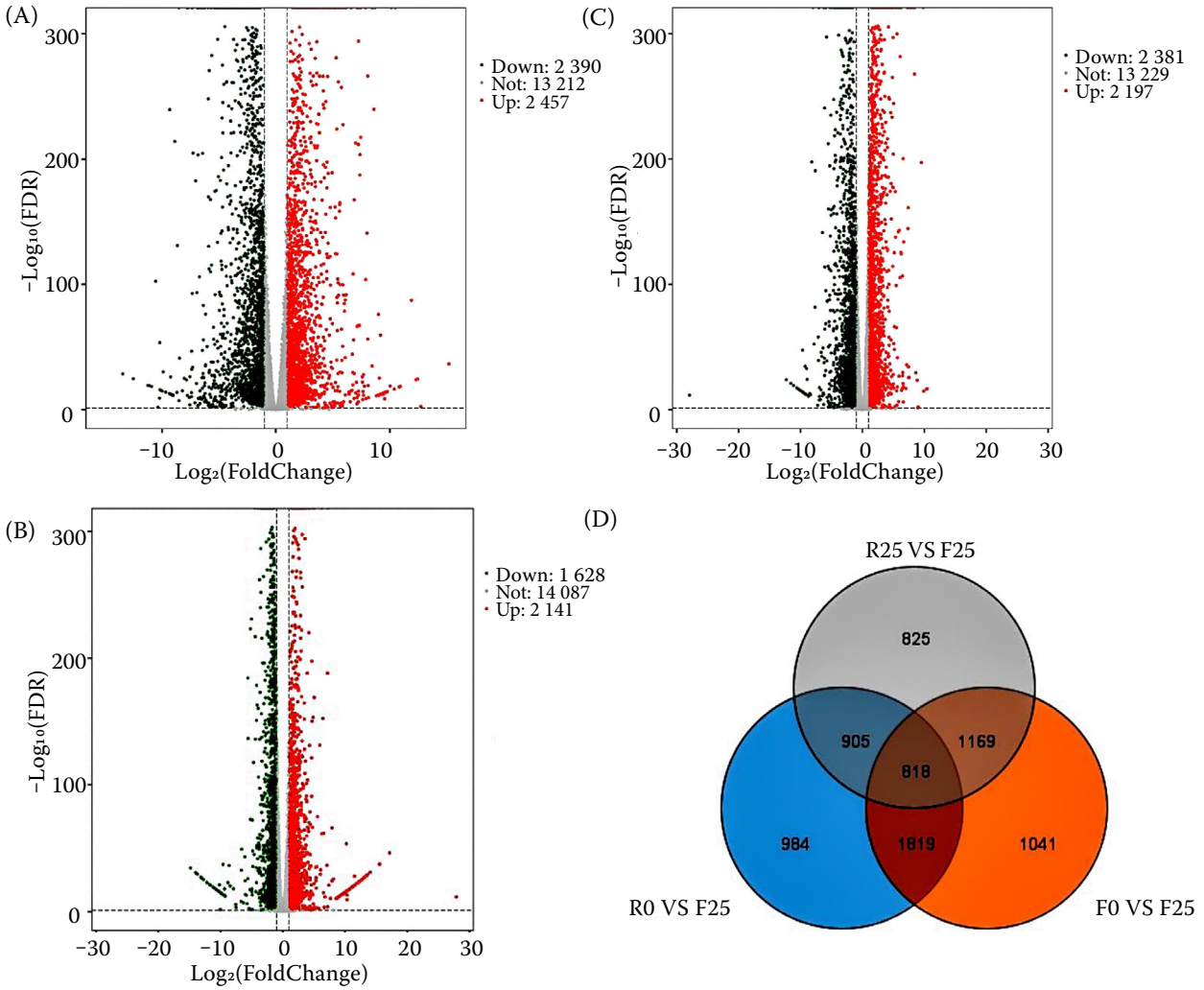

Figure 2. Transcriptome analysis of melon infected by *Phelipanche aegyptiaca*

(A–C) Transcriptome analysis of both the resistant line K16 and the susceptible line K27 following 25 days of *P. aegyptiaca* inoculation; the experimental groups include R0 (K16 control), R25 (K16 25 days post-inoculation with *P. aegyptiaca*), F0 (K27 control), and F25 (K27 25 days post-inoculation with *P. aegyptiaca*); differentially expressed genes (DEGs) are visualised with volcano plots (FDR < 0.01; fold change > 2); (D) Venn diagrams illustrating the overlap of DEGs from F0 vs F25, R0 vs R25 and R25 vs F25

pressed genes (DEGs) in response to *P. aegyptiaca* infestation, we conducted a thorough GO functional annotation analysis on DEGs identified in both the resistant line K16 and the susceptible line K27 (Figure 2D). Our objective was to identify common and significantly enriched GO annotations (p-value ≤ 0.05) (Figure 3). Within biological process, five entries demonstrated significant enrichment, two of which were linked to nitrogen transport (nitrate assimilation, GO: 0042128; nitrate transmembrane transport, GO: 0015706). The remaining three entries were associated with responses to karrikin (GO: 0080167), chitin (GO: 0010200), and hydrogen peroxide catabolic processes (GO: 0042744). Moving to cellular components, five entries showed significant enrichment, with three pertaining to cellular components (plant-type cell wall, GO: 0009505; cell wall, GO: 0005618; plasma membrane, GO: 0005886) and two to extracellular regions (extracellular region, GO: 0005576; apoplast, GO: 0048046). In the molecular function category, eight entries were significantly enriched. Three were associated with binding, three with enzyme activity (including oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, GO: 0016705; peroxidase ac-

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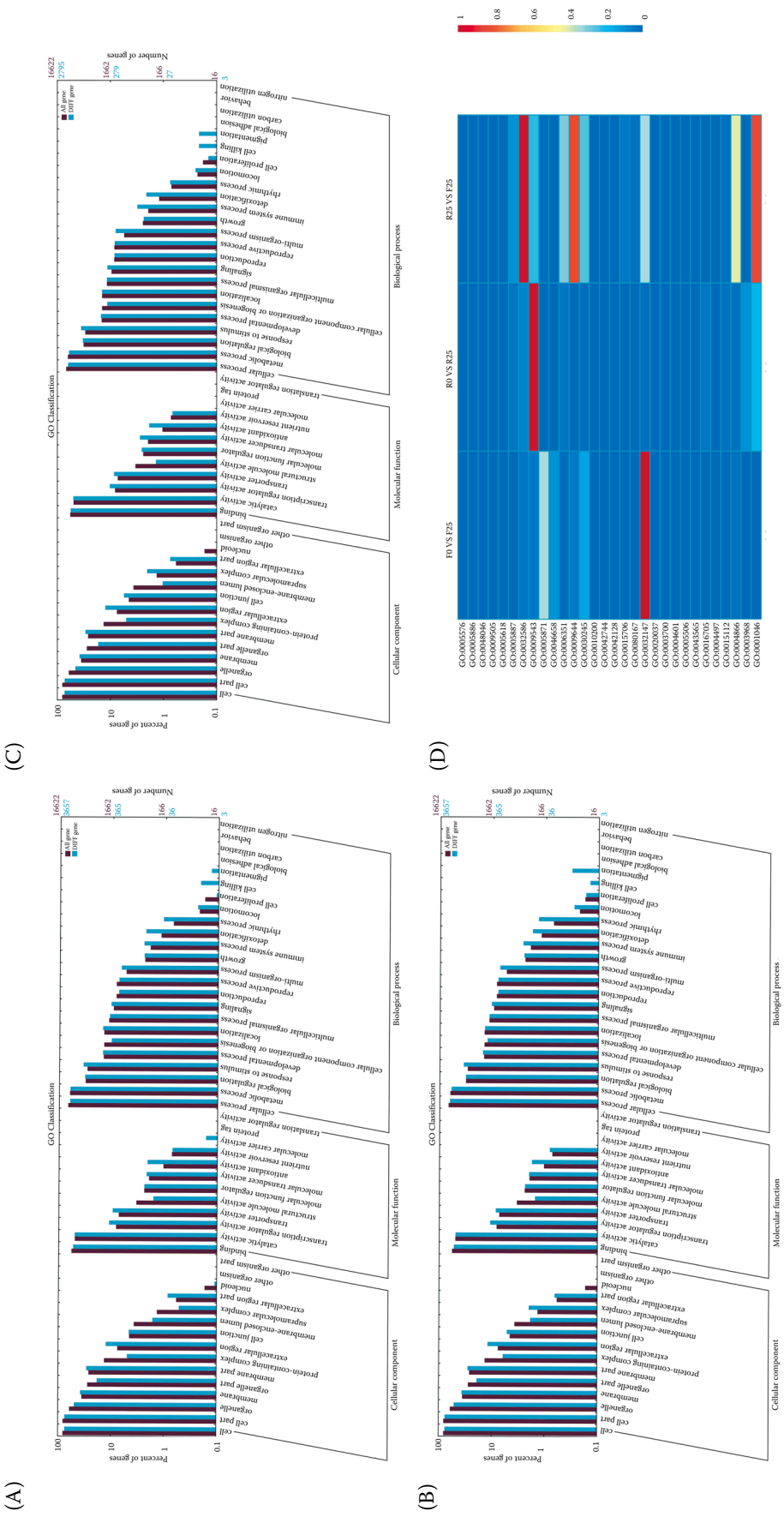

Figure 3. Functional categories of differentially expressed genes in response to *Phelipanche aegyptiaca*

Differentially expressed genes (DEGs) were identified through transcriptome analysis of both the resistant line K16 and the susceptible line K27 following 25 days of *P. aegyptiaca* inoculation [false discovery rate (FDR) < 0.01; fold change > 2]; experimental groups include R0 (K16 control), R25 (K16 25 days post-inoculation with *P. aegyptiaca*), F0 (K27 control), and F25 (K27, 25 days post-inoculation with *P. aegyptiaca*); gene ontology (GO) term enrichment analysis was conducted on DEGs from F0 vs F25 (A), R0 vs R25 (B), and R25 vs F25 (C); the *q*-value of each GO term is represented by a scaled color code (D)

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tivity, GO: 0004601; monooxygenase activity, GO: 004497), and two with DNA-binding transcription factor activity (GO: 003700) and nitrate transmembrane transporter activity (GO: 0015112). These findings provide a comprehensive understanding of the functional roles of DEGs in melon varieties in response to *P. aegyptiaca*, shedding light on key biological processes, cellular components, and molecular functions pivotal for the plant's defence mechanisms.

KEGG enrichment of DEGs upon *P. aegyptiaca* infestation. To unravel the functional pathways influenced by differentially expressed genes (DEGs) in response to *P. aegyptiaca* infestation, we conducted KEGG functional enrichment analysis on DEGs identified in both the resistant line K16 and the susceptible line K27 (Figure 2D), (q-value ≤ 0.05) (Figure 4). In the DEGs from the susceptible line K27 (F0 vs. F25), 13 significantly enriched pathways emerged, with plant hormone signal transduction (map04075) being the sole pathway associated with environmental information processing. At the same time, the remaining 12 were exclusively linked to metabolic pathways (Figure 4A). A total of 960 genes were annotated, with plant hormone signal transduction (ko: map04075) having the highest annotation count at 294 genes, followed by phenylpropanoid biosynthesis (map00940) with 181 genes. The DEGs from the resistant line K16 (R0 vs. R25) significantly enriched 14 pathways spanning metabolism, organic systems, and environmental information processing (Figure 4B). Notably, the MAPK signaling pathway (map04016), phenylpropanoid biosynthesis (map00940), plant-pathogen interaction (map04626), plant hormone signal transduction (map04075), and starch and sucrose metabolism (map00500) each annotated more than 100 genes. Plant hormone signal transduction (map04075) had the highest annotation count, featuring 284 genes. In the DEGs from the comparison of R25 and F25 (R25 vs F25), 22 pathways exhibited significant enrichment (Figure 4C). Within these pathways, 435 differentially expressed genes were identified. Phenylpropanoid biosynthesis (map00940) led with the highest annotation count, featuring 75 genes, followed closely by plant hormone signal transduction (map04075) and starch and sucrose metabolism (map00500), with 61 and 31 genes annotated, respectively.

Upon deeper investigation, we identified six metabolic pathways significantly enriched in all three comparison groups (Figure 4). While plant hormone signaling transduction (map04075) was uniquely associated with the processing of the environmental cues, the remaining five pathways, phenylpropanoid biosynthesis (map00940), alpha-Linolenic acid metabolism (map00592), linoleic acid metabolism (map00591), cyanoamino acid metabolism (map00460), and nitrogen metabolism (map00910), were all related to metabolism. Phenylpropanoid biosynthesis and plant hormone signal transduction were particularly noteworthy, with 29 and 18 annotated differentially expressed genes, respectively. Notably, the annotated genes associated with plant hormone signal transduction played crucial roles in pathways related to cytokinin (CK), abscisic acid (ABA), salicylic acid (SA), brassinosteroid (BR), jasmonic acid (JA), and gibberellin (GA) synthesis. Among these, eight DEGs were annotated for auxin biosynthesis, making it the most represented pathway. Additionally, three were annotated in the SA synthesis pathway. These findings underscore the significance of phenylpropanoid biosynthesis and hormone signaling in response to *P. aegyptiaca* infestation in melon.

Screening of candidate genes for resistance to *P. aegyptiaca* parasitism in melon. The six metabolic pathways consistently enriched in the three groups (Figure 4) led to the identification of 59 genes (Figure 5A). A subsequent screening process focused on these genes to pinpoint differentially expressed candidates with opposite expression patterns between the susceptible line K27 (F0 vs F25) and the resistant line K16 (R0 vs R25). This meticulous screening effort resulted in identifying nine candidate genes demonstrating the potential to play a crucial role in conferring resistance to *P. aegyptiaca* parasitism (Table 2).

Upon thorough analysis, nine candidate genes (Figure 5B, Table 2) were scrutinised for their potential roles in resistance to *P. aegyptiaca* parasitism. Among these, MELO3C017382, identified in the cyanuric acid metabolism and phenylpropanoid biosynthesis pathway, along with MELO3C015359 and MELO3C019540 in phytohormone signaling, and MELO3C007884 in the phenylpropanoid biosynthesis pathway, exhibited significant up-regulation and expression in the *P. aegyptiaca* parasitised line K27. In contrast, these genes were significantly down-regulated and expressed in the *P. aegyptiaca* parasitism-resistant line K16. Conversely, MELO3C008186, MELO3C017690, MELO3C018719, MELO3C025684,

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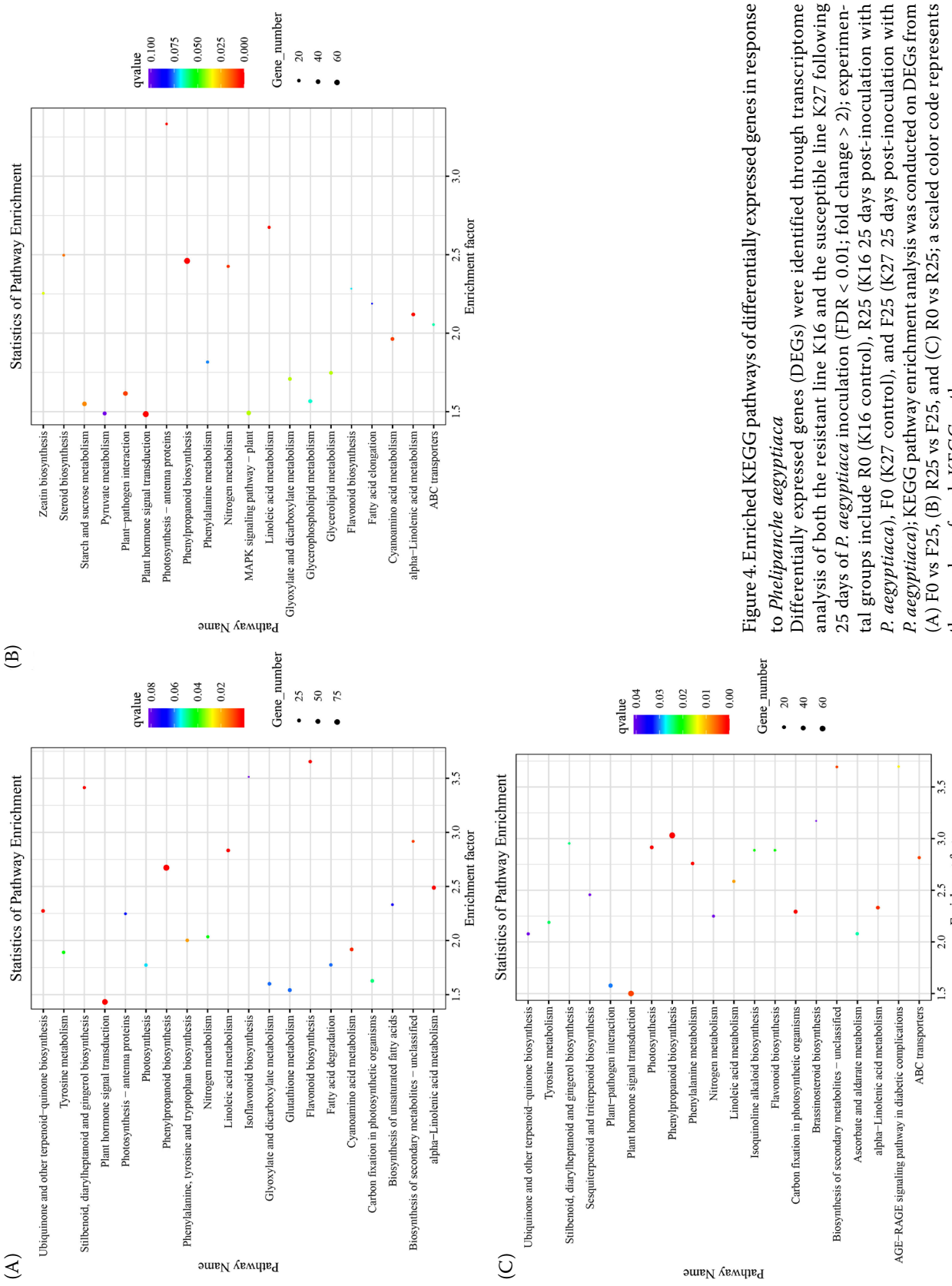

Figure 4. Enriched KEGG pathways of differentially expressed genes in response to *Phelipanche aegyptiaca*

Differentially expressed genes (DEGs) were identified through transcriptome analysis of both the resistant line K16 and the susceptible line K27 following 25 days of *P. aegyptiaca* inoculation (FDR < 0.01; fold change > 2); experimental groups include R0 (K16 control), R25 (K16 25 days post-inoculation with *P. aegyptiaca*), F0 (K27 control), and F25 (K27 25 days post-inoculation with *P. aegyptiaca*); KEGG pathway enrichment analysis was conducted on DEGs from (A) F0 vs F25, (B) R25 vs F25, and (C) R0 vs R25; a scaled color code represents the *q*-value of each KEGG pathway

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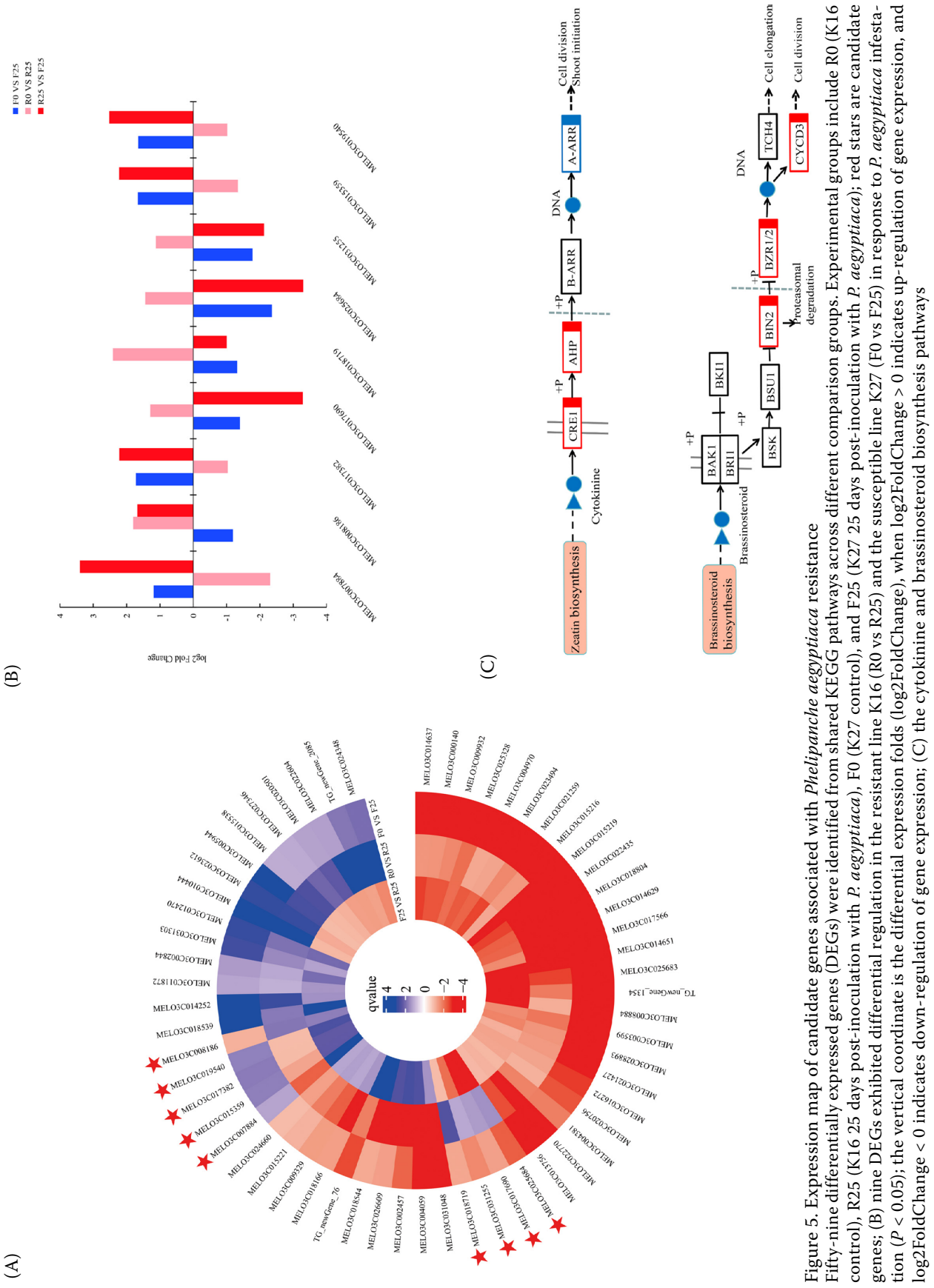

Figure 5. Expression map of candidate genes associated with *Phelipanche aegyptiaca* resistance
Fifty-nine differentially expressed genes (DEGs) were identified from shared KEGG pathways across different comparison groups. Experimental groups include R0 (K16 control), R25 (K16 25 days post-inoculation with *P. aegyptiaca*), F0 (K27 control), and F25 (K27 25 days post-inoculation with *P. aegyptiaca*); red stars are candidate genes; (B) nine DEGs exhibited differential regulation in the resistant line K16 (R0 vs R25) and the susceptible line K27 (F0 vs F25) in response to *P. aegyptiaca* infestation ($P < 0.05$); the vertical coordinate is the differential expression folds (log2FoldChange), when log2FoldChange > 0 indicates up-regulation of gene expression, and log2FoldChange < 0 indicates down-regulation of gene expression; (C) the cytokinine and brassinosteroid biosynthesis pathways

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Table 2. Candidate genes involved in the resistance of melon to *Phelipanche aegyptiaca* parasitism

Gene ID	Gene Position		CDs	Size(aa)	Target gene annotation	Arabidopsis
	Start	End (+/−)				
MELO3C007884	5998846	6001977 (+)	1 530	509	Cytochrome P450 protein	AT4G36220.1
MELO3C008186	1701929	1702904 (−)	783	245	Peroxidase	AT5G14130.1
MELO3C017382	23788311	3793535 (−)	1 887	628	Beta-glucosidase BoGH3B-like	AT5G20950.1
MELO3C017690	24238516	24239946 (−)	1 344	447	HXXXD-type acyl-transferase family protein	AT3G26040.1
MELO3C018719	2350805	2352997 (−)	945	314	Peroxidase	AT5G05340.1
MELO3C025684	24848416	24849761 (+)	963	320	Peroxidase	AT5G05340.1
MELO3C031255	20737855	20739845 (+)	1 581	526	Cytochrome P450 family cinnamate 4-hydroxylase	AT2G30490.1
MELO3C015359	1029369	1032563 (+)	456	151	Histidine-containing phosphotransfer protein, putative	AT3G21510.1
MELO3C019540	11643274	11645306 (+)	1 131	375	Cyclin D3-1	AT4G34160.1

CDs – coding sequences

and MELO3C031255, involved in the biosynthesis pathway of phenylpropanoid substances, displayed a distinct expression pattern. These genes were significantly downregulated in the parasitical line K27, while they exhibited significant upregulation in the anti-parasitical line K16. Based on these expression patterns, a hypothesis emerged suggesting that the identified genes may play pivotal roles in the resistance of melon to *P. aegyptiaca* parasitism. The differential regulation of these genes in response to parasitic infestation hints at their potential involvement in melon's defence mechanisms, providing a foundation for further investigations and functional validations to elucidate their precise roles in conferring resistance to *P. aegyptiaca*.

DISCUSSION

The prevalence of *P. aegyptiaca* has emerged as a significant challenge impeding the robust growth of Xinjiang's melon industry. Lacking autonomous photosynthetic organs, *P. aegyptiaca* depends on its host for nutrients, resulting in compromised host growth and, in severe cases, crop failure (Cubero & Hernández 1991; Velasco et al. 2012; Gutiérrez et al. 2013; Shi & Zhao 2020). Traditional genetic breeding methods for cultivating *P. aegyptiaca*-resistant melon varieties are time-consuming and often yield suboptimal results. Therefore, there is an urgent need to explore genes associated with *P. aegyptiaca* resistance, understand the relevant mechanisms, and employ molecular markers or gene editing techniques to develop new, resistant melon varieties. In this study, we screened and obtained the highly *P. aegyptiaca*-resistant melon line K16. By subjecting both K16 and the *P. aegyptiaca*-sensitive line K27 to *P. aegyptiaca* infestation, we analysed the differential responses to *P. aegyptiaca* using RNA-Seq technology. We aim to elucidate target genes, providing a genetic foundation for subsequent *P. aegyptiaca*-resistant melon breeding.

Plant hormones play a crucial role in mediating the response and adaptation of plants to the environment. Interactions between different hormone signaling pathways regulate various aspects of plant growth, development, and environmental adaptability. Parasitic plant infestations can induce transcriptional reprogramming of multiple hormone signaling pathways in the host. For instance, host plants' insensitivity to cytokinin (CK) has been linked to increased parasitic numbers (Clarke et al. 2020). The parasitic plant (Ogawa et al. 2022) recognise strigolactones released by host plants, promoting parasitism and inhibiting host branching (Gomez-Roldan et al. 2008; Galili et al. 2021). Inhibitors of strigolactone biosynthesis effectively reduce the parasitic plant seed germination and control their growth (Ito et al. 2011; Ito et al. 2013). Additionally, ABA and ethylene can regulate seed dormancy (Wiseglass et al. 2019), while carotenoid biosynthesis inhibitor fluoroether can induce seed germination (Yao et al. 2016). In this study, we identified the gene MELO3C019540, encoding a D-type cyclin (CYCD3) in the pathway associated with BR

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biosynthesis. CYCD3 regulates cambial cell proliferation and secondary growth (Collins et al. 2015). This gene was upregulated in the susceptible line K27 and downregulated in the resistant line K16 (Figure 5B). We hypothesise that, upon parasitisation by *P. aegyptiaca*, the susceptible melon line enhances its resistance by promoting cell proliferation through CYCD3, which helps plants to recover and adapt quickly in adversity. Furthermore, we identified the gene MELO3C015359, encoding a histidine phosphotransfer protein involved in CK biosynthesis. This gene was upregulated in the susceptible line K27 and downregulated in the resistant line K16. Previous studies in rice demonstrated that histidine phosphotransfer proteins (AHP) OsAHP1 and OsAHP2 play a positive regulatory role in cytokinin signaling and have different functions in salt and drought tolerance (Sun et al. 2014).

Phenylpropanoid biosynthesis and cyanoamino acid metabolism are essential processes that produce numerous secondary metabolites, aiding plants in resisting various stress conditions (Dong & Lin 2021). Phenylpropanoids are pivotal in plant response to biotic and abiotic stimuli (La Camera et al. 2004). Lignification, closely related to phenylpropanoid metabolism, strengthens host plant cell walls, providing resistance against pathogen infections (Sonbol et al. 2009). β-Glucosidase, an important component of cellulase, participates in the breakdown of cell walls, leading to a decrease in the ability of cells to resist diseases. This study identified several differentially expressed genes, collectively participating in cyanogenic amino acid metabolism and phenylpropanoid biosynthesis. Among them, MELO3C017382, encoding β-glucosidase, was co-enriched incyanogenic amino acid metabolism and phenylpropanoid biosynthesis. It was upregulated in susceptible material K27 and downregulated in resistant material K16. The cell wall (GO:0005618, GO: 0009505) was annotated in GO enrichment analysis. It is speculated that MELO3C017382 plays an important role in the secondary metabolism involved in the interaction between melon and *P. aegyptiaca*.

The cytochrome P450 enzyme (CYP) within the phenylpropanoid biosynthesis pathway plays a crucial role in producing reactive oxygen species enhancing plant stress resistance (Xu et al. 2022). In tomatoes, CYP genes (Solyc06g073570, Solyc06g074180, Solyc06g074420) were found to be associated with the strigolactone (SL) pathway, improving tomato resistance to *P. aegyptiaca* (Bai et al. 2020). In this study, we identified three genes related to reactive oxygen species (MELO3C018719, MELO3C025684, MELO3C008186) and two genes related to CYP (MELO3C007884, MELO3C031255). MELO3C007884 encodes cytochrome P450, while MELO3C031255 encodes cytochrome P450 cinnamate 4-hydroxylase (C4H). The three genes related to reactive oxygen species and MELO3C031255 were upregulated in the resistant line K16 and down-regulated in the susceptible line K27. It is hypothesised that MELO3C031255 regulates CYP secretion during the interaction between *P. aegyptiaca* and melons, and CYP, in turn, regulates the expression of three genes involved in reactive oxygen species to enhance resistance against *P. aegyptiaca*. ZmCYP706C37, a member of the cytochrome P450 family in maise, is involved in the biosynthesis pathway of melatonin. Its activity affects the secretion of strigolactone by maise roots, which can change strigolactone composition and reduce Striga germination and infection on maise plants (Li et al. 2023). This study identifies MELOC007884 as a candidate gene encoding cytochrome P450 protein, suggesting its potential involvement in the interactions between *P. aegyptiaca* and melon.

Nitrogen, an indispensable nutrient for plant growth and development, plays a crucial role in the plant's ability to withstand various environmental challenges. Previous studies have reported interactions between Cuscuta and its hosts involving nitrogen and nitrogen system signaling (Zhang et al. 2021b). It is speculated that similar interactions may exist between *P. aegyptiaca* and the host. This study enriched nitrogen-related pathways multiple times, suggesting their significance in the interaction between *P. aegyptiaca* and melon. The nitrate transporter BnNRT2 coordinates the absorption and transport of nitrate in rapeseed, responding to cues from multiple stress response pathways (Remans et al. 2006). The GO enrichment analysis revealed simultaneous enrichment of nitrate assimilation (GO:0042128) and nitrate transmembrane transport (GO: 0015706). Furthermore, KEGG enrichment indicated the involvement of multiple nitrate transporters in the interaction.

CONCLUSION

Transcriptomic analysis was conducted on two distinct resistant melon materials, identifying

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818 significantly differentially expressed genes. Through GO functional annotation and KEGG enrichment, a subset of genes was co-expressed significantly in all three comparisons, identifying nine candidate genes. These genes are hypothesised to be associated with melon resistance to *P. aegyptiaca* parasitism. The outcomes of this study offer valuable insights and a foundation for understanding the mechanisms involved in *P. aegyptiaca* parasitisation in melons and contribute to the breeding efforts aimed at developing *P. aegyptiaca*-resistant varieties.

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