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Article

Endophytic *Beauveria bassiana* of Tomato Resisted the Damage from Whitefly *Bemisia tabaci* by Mediating the Accumulation of Plant-Specialized Metabolites

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ABSTRACT: *Beauveria bassiana* acts as an endophytic fungus that controls herbivorous pests by stimulating plant defenses and inducing systemic resistance. Through multiomics analysis, 325 differential metabolites and 1739 differential expressed genes were observed in tomatoes treated with *B. bassiana* by root irrigation; meanwhile, 152 differential metabolites and 1002 differential genes were observed in tomatoes treated by local leaf spraying. Among the upregulated metabolites were α-solanine, 5-*O*-caffeoylshikimic acid, clerodendrin A, and peucedanin, which demonstrated anti-insect activity. These differential metabolites were primarily associated with alkaloid biosynthesis, flavonoid biosynthesis, and tryptophan metabolism pathways. Furthermore, the gene silencing of UDP-glucose:sterol glucosyltransferase, a gene involved in α-solanine synthesis, indicated that *B. bassiana* could inhibit the reproduction of whiteflies by regulating α-solanine. This study highlighted the ability of *B. bassiana* to modulate plant secondary metabolites and emphasized the significance of understanding and harnessing multitrophic interactions of endophytic *B. bassiana* for sustainable agriculture.

KEYWORDS: *Beauveria bassiana, tomato, Bemisia tabaci, plant secondary metabolites, metabolomic, transcriptomic*

INTRODUCTION

Plants and their associated microorganisms have a mutually beneficial relationship. These microorganisms, known as endophytes, reside within plant tissues and provide several benefits without causing harm. One key benefit is their role in enhancing the adaptation and resistance of a plant to various stresses.[1,2] Endophytes activate the defense mechanisms of a plant, aiding in the fight against pathogens and herbivores.[3−5] Furthermore, endophytes play a crucial role in nutrient acquisition, including helping plants acquire essential nutrients from the soil[6] and promoting plant growth and overall health.[7,8] The presence of endophytes also contributes to the co-evolution of plants and other organisms in the ecosystem.[9−11]

Entomopathogenic fungi (EPF) belong to the order Hypocreales and are renowned for their insect-infecting and killing abilities. They have been extensively used in agricultural settings as biocontrol agents to combat pests.[11] However, their effectiveness can be compromised by various environmental factors in outdoor environments, including moisture, temperature, precipitation, and ultraviolet (UV) radiation.[12,13] To overcome these challenges, researchers have investigated the potential of EPF to establish endophytic relationships with plants. For instance, studies have demonstrated that EPF species, such as *Beauveria* (Cordycipitaceae) and *Metarhizium* (Clavicipitaceae), can colonize various crops, such as tomatoes, cotton, potatoes, corn, legumes, and wheat.[14] Moreover, recent research has shed light on several mechanisms by which endophytic EPF can reduce pest abundance, fecundity, survival, and weight. These mechanisms include feeding on EPF hyphae, mycosis, herbivore-induced plant volatiles (HIPVs), kairomones, fungal secondary metabolites, and plant defense induction.[15] This innovative approach presents a promising avenue for investigating the ecology of EPF and exploring their potential as sustainable biocontrol agents in agriculture.[16,17]

Plants produce specialized bioactive compounds, which are crucial and diverse elements of the plant defense system.[18] These specific metabolites aid plants in responding to herbivore attacks by minimizing damage and preserving their overall fitness.[19,20] One group of plant secondary metabolites (PSMs), known as *Solanum* steroidal glycoalkaloids (SGAs), is primarily found in the Solanaceae family.[21] SGAs possess unique structures and exhibit significant pharmacological activities, including cytotoxic, antimicrobial, and anticholesterol properties. Additionally, some SGAs, such as α-tomatine, dehydrotomatine, and α-solanine, are well-known toxins.[22] Given the expanding scope of research, more scholars have recently started to examine PSMs. For example, a previous study evaluated the changes in 11 benzoxazinoids in wheat leaves and 12 flavonoids in bean leaves after treatment with EPF.[23] However, there is still a lack of comprehensive understanding regarding the overall pattern of PSM modifications in plant tissues following EPF colonization.

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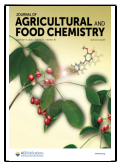

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Moreover, there is no current evidence suggesting that the colonization of EPF, specifically *Beauveria bassiana*, can enhance resistance to pests by inducing the accumulation of SGAs.

In this study, we assessed the survival rate and fertility of whiteflies after feeding on tomatoes that had been treated with *B. bassiana*. The results demonstrated significant suppression of whitefly reproduction when tomatoes were treated with *B. bassiana* through root irrigation. To gain insight into the underlying mechanisms, we conducted metabolomic and transcriptomic analyses to investigate the internal changes that occurred in tomatoes following the inoculation with *B. bassiana*. Our findings revealed that colonization by *B. bassiana* led to alterations in the accumulation of PSMs, thereby enhancing plant resistance at both the molecular and chemical levels. Moreover, we employed virus-induced gene silencing (VIGS) technology to confirm the role of *B. bassiana* in inhibiting whitefly propagation by inducing the synthesis and accumulation of α-solanine. Overall, our study emphasizes the importance of comprehending the regulation of PSMs in multitrophic interactions, which will aid in the future development of environmentally friendly pest control methods in agriculture. By harnessing the potential of beneficial fungi, such as *B. bassiana*, we can strive toward sustainable and effective agricultural practices.

MATERIALS AND METHODS

Plants, Insects, and Fungal Strains. This study utilized surface-sterilized seedlings of *Solanum lycopersicum* cv. Hezuo 903 for experimentation. These seedlings were sown in nutrient soil that had undergone high-temperature sterilization. The entire process was conducted under controlled conditions, which included maintaining a temperature of 26 ± 1 °C, relative humidity of 65 ± 5%, and a light/dark cycle of 14/10 h.[24] To ensure consistency, tomato plants with five fully expanded leaves were selected for the experiments and watered every 3 days.

The *Bemisia tabaci* Mediterranean (MED) population was maintained on tomato plants (cv. Hezuo 903) in a climate chamber with a temperature of 26 ± 1 °C, relative humidity of 65 ± 5%, and a light/dark photoperiod of 14/10 h. Only newly emerged female adults (less than 48 h old) were used for the experiments.

B. bassiana strain Bb252, isolated from *Chilo suppressalis* (Walker), was cultivated on potato dextrose agar (PDA) medium in the dark at 26 ± 1 °C. To prepare the strain for experimentation, it was diluted with 0.05% Tween 80.[24]

Plant Treatment. The 10 mL aliquot of a 1×10^8 conidial mL^{-1} suspension of *B. bassiana* was used for local leaf spraying (BbL) and root irrigation (BbR). Control plants were sprayed with a 10 mL solution of 0.05% Tween 80. The treated plants were kept at 26 ± 1 °C and 65 ± 5% relative humidity under a 14 h light/10 h dark (L/D) photoperiod.

Whitefly Performance Assays. To investigate the impact of *B. bassiana* colonization on the survival and fecundity of *B. tabaci*, we selected tomatoes inoculated by local leaf spraying and root irrigation for 7 days and newly emerged female adults of *B. tabaci* to perform related experiments in clip cages (3 cm in diameter). Two leaves at the same position without direct contact with the spore suspension were selected from each tomato plant for the following experiment. Each leaf was enclosed in a cage containing 4−5 female whiteflies. After 3 and 5 days, we counted the surviving adults and the eggs laid by each adult in the chip cages for each treatment using a microscope.

Metabolomics Analysis. Leaf samples were collected and analyzed using untargeted metabolomics via ultra-high-performance liquid chromatography−quadrupole time-of-flight mass spectrometry (UHPLC−QTOF−MS) after a 7 days treatment with *B. bassiana*. The Biomarker Technologies Corporation conducted the analysis. Differential metabolites were identified by combining the fold change and the variable importance in the projection (VIP) value. The fold change threshold was set at either ≥2.0 or ≤0.5, with a VIP value of >1.[25]

Transcriptional Profiling. Leaf samples were sent to the Biomarker Biotechnology Company for RNA extraction, cDNA library construction, and RNA-Seq analysis. To generate sequencing libraries, the NEBNext Ultra RNA Library Prep Kit for Illumina [New England Biolabs (NEB), Ipswich, MA, U.S.A.] was utilized following the recommendations of the manufacturer. Index codes were added to attribute sequences to each respective sample. The clustering of the index-coded samples was carried out on a cBot Cluster Generation System using TruSeq PE Cluster Kit v4-cBot-HS (Illumia) following the instructions of the manufacturer. Once cluster generation was complete, library preparations were sequenced on an Illumina platform, which generated paired-end reads. To further process the raw reads, a bioinformatic pipeline tool, BMKCloud (www.biocloud.net), was utilized through the online platform. Differentially expressed genes were identified using the fold change criterion (≥2) and the false discovery rate (FDR) threshold (<0.01).

RNA Extraction and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) Analysis. Total RNA extraction from tomato leaves was performed using the PrimeScript RT-PCR Kit (TaKaRa Bio, Inc., Shiga, Japan) for reverse transcription into first-strand cDNA. Quantitative polymerase chain reaction (qPCR) was conducted using NovoStart SYBR qPCR SuperMix Plus (Novoprotein, Shanghai, China). Internal normalization controls were established using the *actin* gene and *UBI3* gene. Gene expression levels were determined using the $2^{-\Delta\Delta C_t}$ method. Specific primers for qPCR can be found in Table S1 of the Supporting Information.

Exogenous Metabolite Treatments. A stock solution of the α-solanine standard was prepared by diluting dimethyl sulfoxide (DMSO) to a concentration of 1 mM. This stock solution was further diluted with deionized water (ddH_2O) to create a treatment group with a concentration of 8.39 μM. Pure water was used as the control. For the treatment, 10 mL of the 8.39 μM solution was administered to five fully expanded tomato leaves. After 24 h, both the experimental and control tomatoes were placed in a rearing cage measuring 40 × 40 × 40 cm. Each cage contained 100 2-day-old female whiteflies. The cages were maintained under controlled conditions of 26 ± 1 °C, 65 ± 5% relative humidity, and a 14 h light/10 h dark photoperiod for 3 days. The number of whiteflies on each tomato plant was observed daily, and after 3 days, the number of eggs was counted under a microscope. A total of 10 similar-sized leaves were selected from each tomato plant, and each treatment consisted of three replicates.

Virus-Induced Gene Silencing (VIGS). We used the tobacco rattle virus (TRV) vectors and identified the target regions of the UDP-glucose:sterol glucosyltransferase (*SlSGT2* and *SlSGT3*) genes using the SGN VIGS tool.[26] The TRV-mediated VIGS system was employed to silence *SlSGT2* and *SlSGT3*, with the pTRV1+TRV:00 group as the negative control and the pTRV1+TRV:*SlPDS* group as the positive control to assess the effectiveness of VIGS. After 4 weeks of injection, *B. bassiana* was subsequently applied to the tomato plants for 7 days, and the gene expression of the silenced gene in the plants was measured using qRT-PCR. For the subsequent experiments, both the silenced and control tomato plants as well as newly emerged *B. tabaci* female adults were selected. Each leaf was enclosed in a cage with a diameter of 3 cm, containing two whiteflies. After 3 days, the number of eggs laid by the adult females in each treatment group was counted using a microscope.

The analysis of α-solanine was carried out using liquid chromatography−tandem mass spectrometry (LC−MS/MS). First, 100 mg of ground powder was weighed and added to 1 mL of an 80% methanol−water solution. The mixture was then shaken and allowed to extract overnight at 4 °C.[27] The α-solanine standard, purchased from SHANGHAI ZZBIO Co., Ltd., was prepared by dissolving 500 mg mL^{-1} of the stock solution in methanol. This was then diluted to concentrations of 5, 2, 1, 0.5, 0.3, and 0.1 mg mL^{-1} to generate a standard curve. The samples were analyzed using an ultra-perform-

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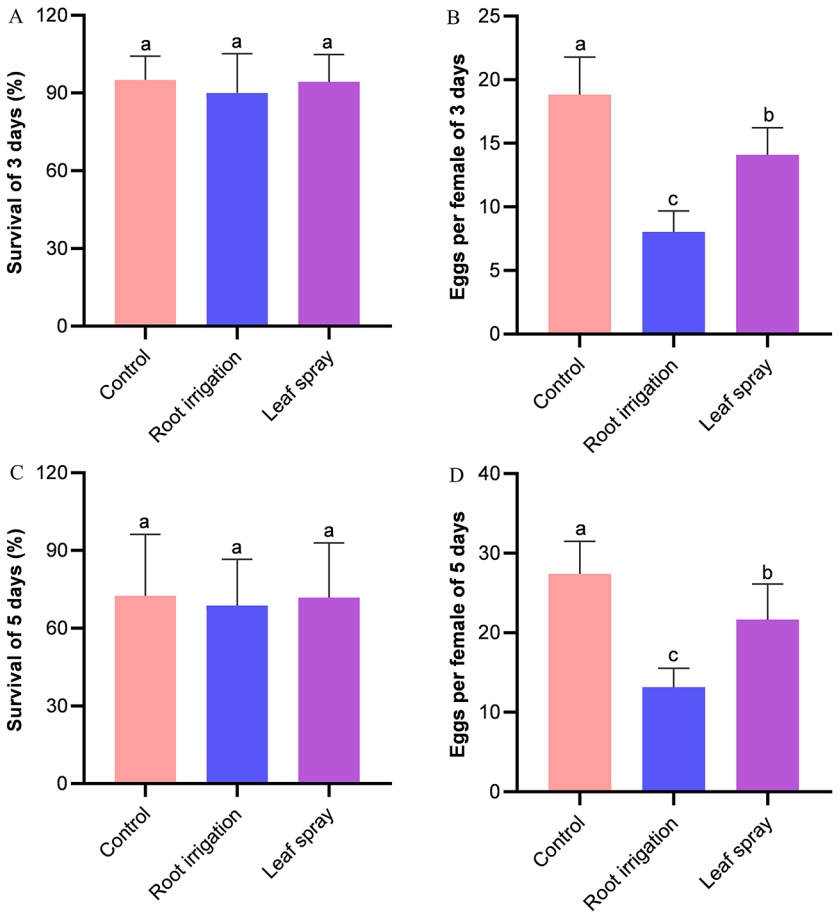

Figure 1. Effects of endophytic *B. bassiana* on the performance of whiteflies: (A) survival rate of adult females at 3 days, (B) fecundity of adult females at 3 days, (C) survival rate of adult females at 5 days, and (D) fecundity of adult females at 5 days. The data are presented as the mean ± standard error (SE) of eight biological replicates. The error bars indicate the standard error. Different letters indicate significant differences among treatments, as determined by one-way ANOVA ($p < 0.05$).

ance liquid chromatography−tandem mass spectrometry (UPLC−MS/MS) system. The mobile phase consisted of solvent A (pure water with 0.1% formic acid) and solvent B (acetonitrile). The sample measurements were taken using a gradient program, starting with 90% A and 10% B. After 2 min, the composition was adjusted to 100% B for 2 min. Then, the composition was readjusted to 90% A and 10% B within 0.10 min and maintained for 2 min. The column oven temperature was set at 40 °C, and an injection volume of 2 μL was used. The effluent was connected to an electrospray ionization (ESI)−triple quadrupole linear ion trap (QTRAP)−mass spectrometry (MS) detector.

Statistical Analysis. Whitefly performance data were analyzed using one-way analysis of variance (ANOVA), with Tukey's test applied to compare the means ($p < 0.05$). Student's *t* test was used to examine differences in the remaining data for this research study. Statistical analyses were conducted using SPSS 20 (IBM Corp., Armonk, NY, U.S.A.). All figures were generated using GraphPad Prism 8.0.2 (GraphPad Software, Inc., La Jolla, CA, U.S.A.).

RESULTS

Effects of Endophytic *B. bassiana* on *B. tabaci* Performance. To evaluate the impact of *B. bassiana*-treated tomatoes on the performance of *B. tabaci*, we conducted a comparison of the survival and fecundity of adult female whiteflies across three different types of tomato plants: those treated with *B. bassiana* via root irrigation, those treated with *B. bassiana* via leaf spraying, and control tomatoes. Following a 3 day feeding period, no significant differences were observed in the survival rate of adult female *B. tabaci* between the *B. bassiana*-treated tomatoes and the control tomatoes ($F_{2,21}$ = 0.4196 and p = 0.6627; Figure 1A). However, we did observe a reduced egg production among the female *B. tabaci* that were fed *B. bassiana*-treated tomatoes through root irrigation compared to those on plants treated with local leaf spraying and the control tomatoes. Additionally, there were significant differences in egg production among the three treatment groups ($F_{2,21}$ = 43.79 and $p < 0.0001$; Figure 1B). These findings remained consistent when assessed after a 5 day feeding period (panels C and D of Figure 1).

***B. bassiana* Colonization Alters Metabolite Profiles in the Tomato Plants.** Figure 1 illustrates that the fecundity of *B. tabaci* was significantly reduced after consuming tomato leaves treated with *B. bassiana*. This study aimed to determine whether this biological phenomenon is the result of effect of *B. bassiana* on the internal substances of tomato leaves. To investigate, we utilized an integrated metabolomics platform that incorporates ultra-high-performance liquid chromatography and tandem mass spectrometry to identify the differential metabolites. We analyzed the leaf tissues of tomato plants 7 days after inoculation with *B. bassiana* and observed significant differences in metabolites compared to the control group. The clustering heatmap of the differential metabolites is presented in Figure 2A, indicating the reproducibility of samples within the root irrigation and control groups. To obtain a

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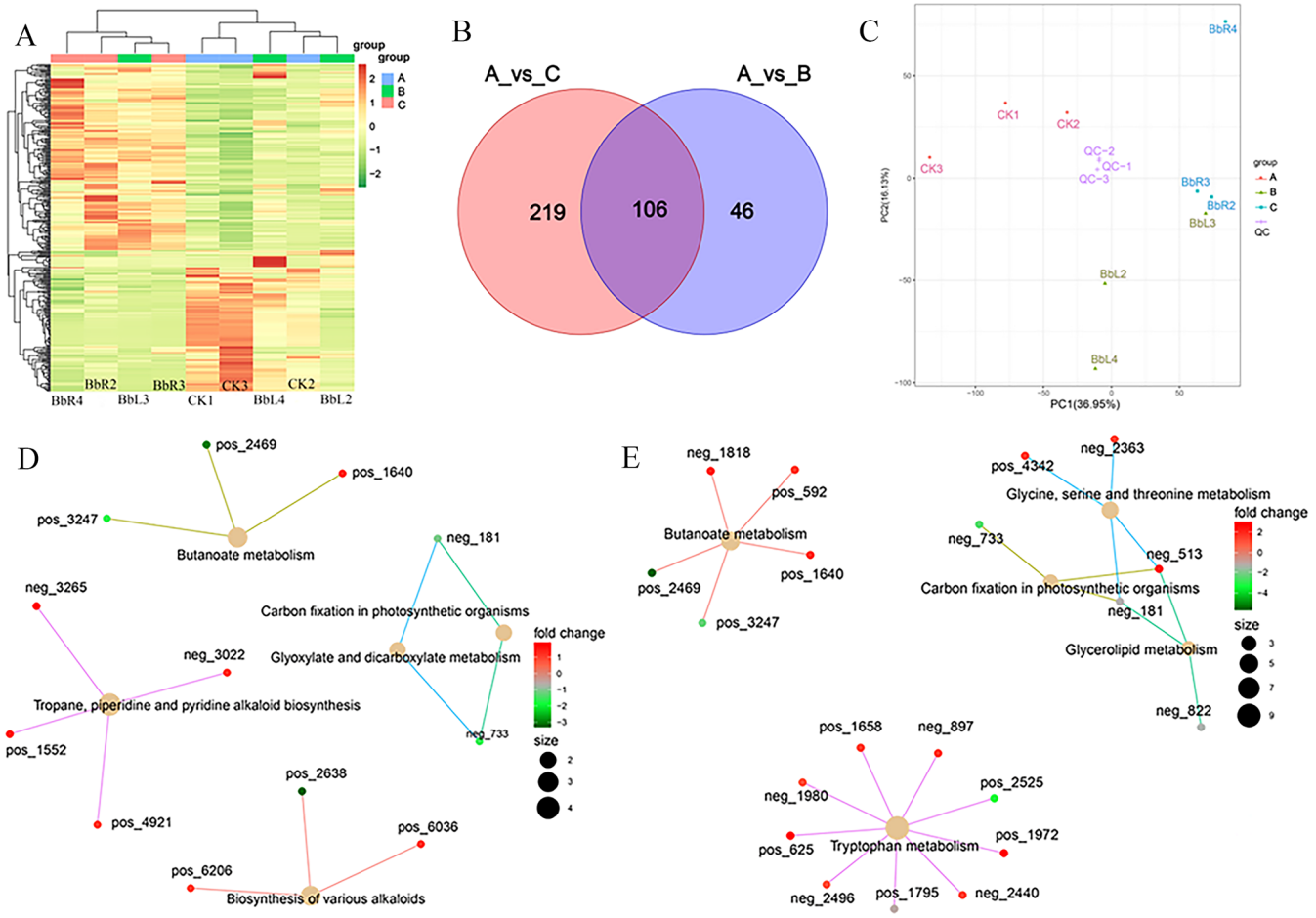

Figure 2. Analysis of differential metabolites between tomatoes treated with *B. bassiana* and the control group: (A) clustering heatmap of quantified differential metabolites, (B) Venn diagram illustrating the differential metabolites, (C) PCA showcasing global metabolite changes in tomato leaves, (D) pathway enrichment analysis of the differential metabolites between tomatoes treated with *B. bassiana* by leaf spraying (BbL) and the control plants, and (E) pathway enrichment analysis of the differential metabolites between tomatoes treated with *B. bassiana* by root irrigation (BbR) and the control plants.

comprehensive understanding of the detected metabolites, principal component analysis (PCA) was performed. The first and second principal components accounted for 36.95 and 16.13% of the total variance, respectively, and were clustered in different areas, indicating distinct metabolic profiles (Figure 2C). Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, we analyzed the enrichment pathways of the major differential metabolites. For local spray-inoculated leaves, the differential metabolites were mainly enriched in the biosynthesis of various alkaloids, butanoate metabolism, and carbon fixation in photosynthetic organisms (Figure 2D). For root-irrigation-inoculated leaves, differential metabolites were enriched in butanoate metabolism and tropane metabolism (Figure 2E). In summary, we observed 152 and 325 differential metabolites in local leaf-sprayed and root-irrigated tomatoes, of which 89 and 193 were upregulated metabolites, respectively (Figure 2B and Tables S2 and S3 of the Supporting Information).

To investigate the potential anti-insect activity of the differentially accumulated metabolites, a study was conducted wherein several commonly studied metabolites were analyzed. The results revealed that 16 of these metabolites exhibited potential anti-insect activity, as depicted in Figure S1 of the Supporting Information. Among these metabolites, six were significantly increased in locally leaf-sprayed and root-irrigated tomatoes. These six metabolites are peucedanin, (+)-dysidea-palaunic acid, monoisobutyl phthalic acid, α-curcumene, ephedrine, and 3,7,4′-tri-*O*-methylquercetin. Notably, α-curcumene has previously been recognized for its insecticidal properties, acting as both a repellent and an insect feeding deterrent.[28] Additionally, when tomatoes were treated with *B. bassiana* via root irrigation, eight metabolites exhibited a significant increase. In particular, clerodendrin A demonstrated antifeedant and growth inhibitory effects on *Spodoptera litura*.[29] Furthermore, α-solanine has been shown to have diverse effects, potentially impairing development, food intake, and reproduction.[30,31]

Dissecting the Transcriptome Profile for *B. bassiana* Colonization of Tomato Plants. We collected tomato leaves that were treated with local leaf spraying and root irrigation for RNA sequencing. A total of 55.85 Gb of clean data was produced by RNA-Seq for nine samples. The GC contents are approximately 43% for each sample, with a Q30 (sequencing error rate of <0.1%) value of greater than 93.76% (Table S4 of the Supporting Information). PCA revealed that colonization of *B. bassiana* had a significant effect on gene expression (Figure 3A). Notably, in the root-irrigation-treated samples, we identified 1739 differentially expressed genes (DEGs) compared to the control, which was significantly higher than the 1002 DEGs observed in the local leaf-spraying treatment

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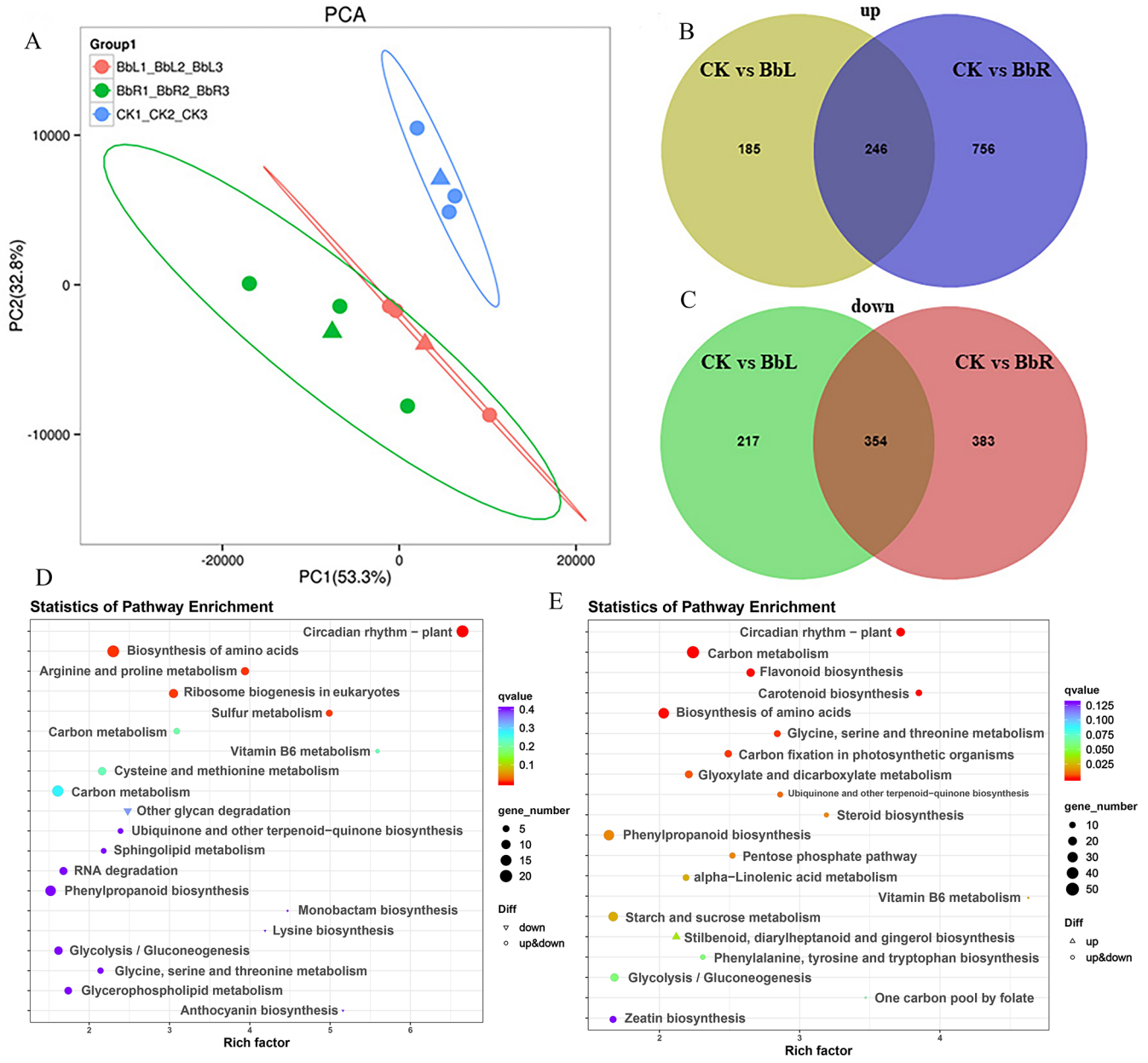

Figure 3. Overall transcriptomic changes in tomato leaves: (A) PCA of DEGs at different samples, Venn diagram depicting the comparison of (B) upregulated DEGs and (C) downregulated DEGs, (D) pathway enrichment analysis of DEGs between tomatoes treated with *B. bassiana* by local leaf spraying (BbL) and the control plants, and (E) pathway enrichment analysis of DEGs between tomatoes treated with *B. bassiana* by root irrigation (BbR) and the control plants.

samples (Tables S5 and S6 of the Supporting Information). Furthermore, we observed a total of 246 upregulated and 354 downregulated common genes in tomatoes treated with *B. bassiana* through both local leaf spraying and root irrigation (panels B and C of Figure 3). To gain further insights, we conducted KEGG pathway enrichment analysis, which revealed significant enrichments in tomato plants treated with local leaf spraying for circadian rhythm of the plant, biosynthesis of amino acids, and arginine and proline metabolism (Figure 3D). Tomato plants treated with root irrigation showed significant enrichment in circadian rhythm of the plant, carbon metabolism, and flavonoid biosynthesis (Figure 3E).

Integration of the Metabolome and Transcriptome To Identify Metabolic Pathways Affected by *B. bassiana*. Metabolo−transcriptomic was employed to investigate the response of tomatoes to colonization by *B. bassiana*, resulting in the identification of four metabolic network diagrams. Our findings demonstrated that colonization by *B. bassiana* stimulated alkaloid biosynthesis in tomatoes (Figure 4). Genes involved in the production of SGAs, such as cholesterol 22-hydroxylase (*GAME7*), 22,26-dihydroxycholesterol 16α-hydroxylase (*GAME11*), and glycoalkaloid metabolism genes (*GAME6* and *GAME4*), were found to be upregulated in tomatoes subjected to root irrigation compared to the control group. Consistent with these results, the levels of metabolites, including α-solanine, γ-chaconine, tomatidine, and tomatine, exhibited an increasing trend in tomatoes treated via root irrigation, with α-solanine showing a particularly significant elevation. Additionally, qPCR analysis revealed a

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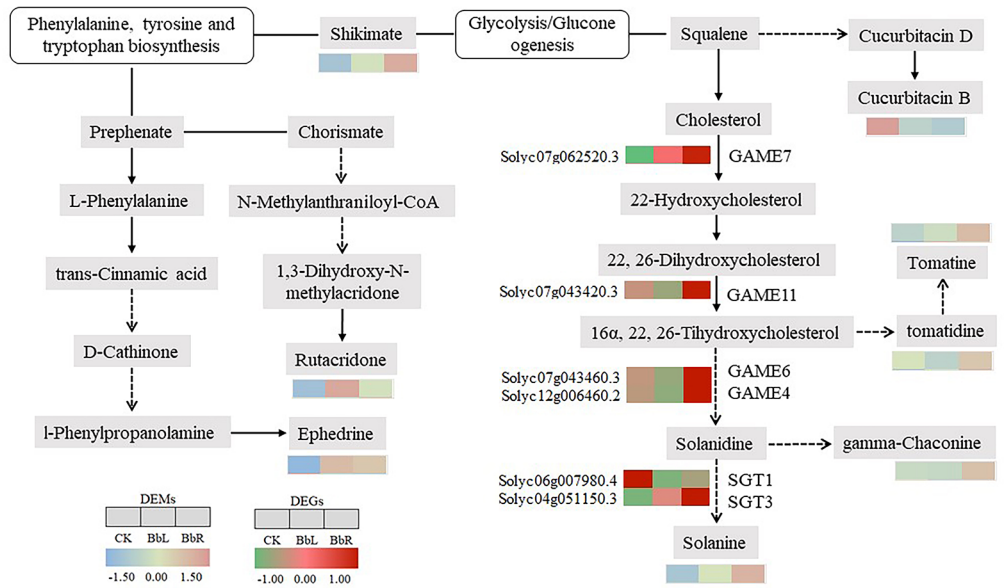

Figure 4. Changes in the biosynthesis of various alkaloid metabolites and genes in *B. bassiana* colonization of tomato leaves compared to the control group. *GAME7*, cholesterol 22-hydroxylase; *GAME11*, 22,26-dihydroxycholesterol 16*α*-hydroxylase; *GAME6* and *GAME4*, glycoalkaloid metabolism genes; and *SGT1* and *SGT3*, UDP-glucose:sterol glucosyltransferase.

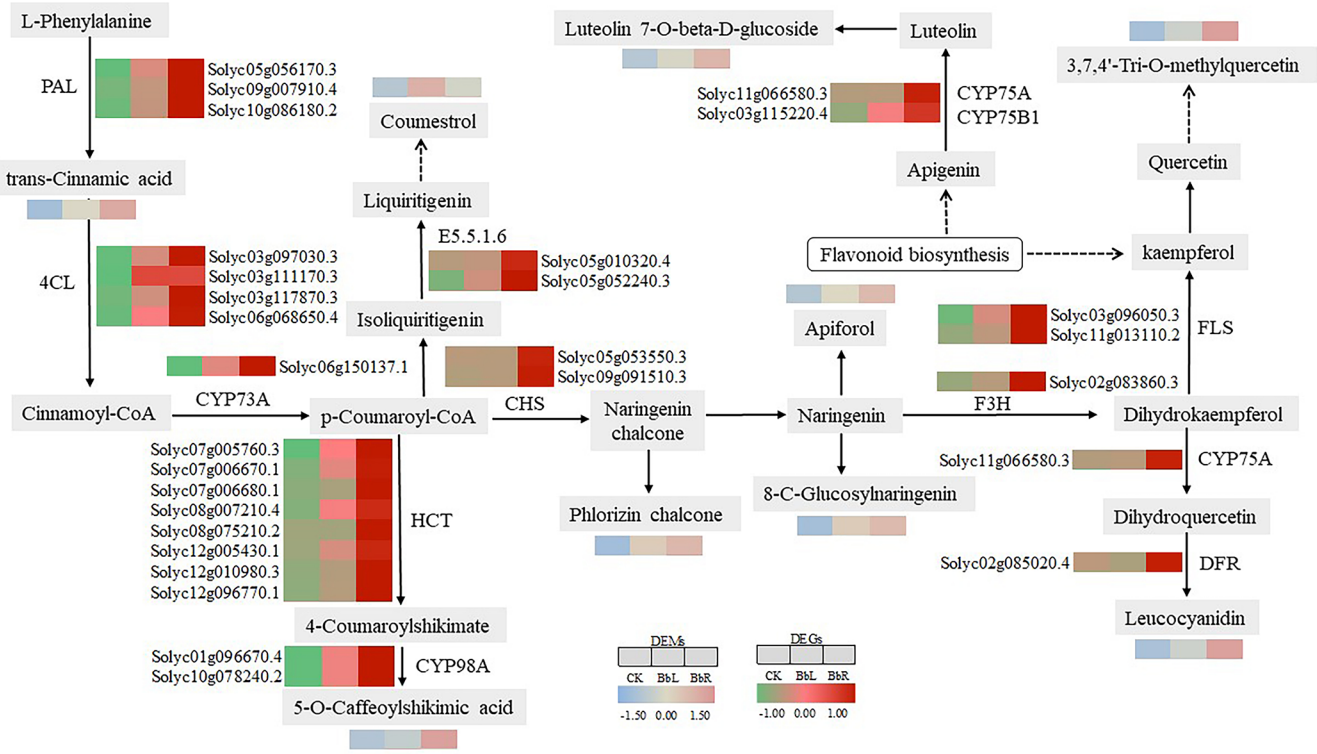

Figure 5. Changes in flavonoid metabolites and the expression of flavonoid biosynthesis genes were investigated in tomato leaves colonized by *B. bassiana* and in control leaves. *PAL*, phenylalanine ammonia lyase; *4CL*, 4-coumarate-CoA ligase; *CYP73A*, *trans*-cinnamate 4-monooxygenase; *HCT*, shikimate *O*-hydroxycinnamoyltransferase; *CYP98A*, 5-*O*-(4-coumaroyl)-D-quinate 3′-monooxygenase; *CHS*, chalcone synthase; *F3H*, naringenin 3-dioxygenase; *CYP75A*, flavonoid 3′,5′-hydroxylase; *DFR*, bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase; *FLS*, flavonol synthase; and *CYP75B1*, flavonoid 3′-monooxygenase.

significant upregulation of genes related to SGA synthesis in tomato leaves treated with *B. bassiana* via root irrigation (Figure S2 of the Supporting Information). SGAs are mainly found in *Solanum* species and possess notable pharmacological activities, such as cytotoxic, antimicrobial, anticholesterol, and toxic properties.[18] Furthermore, *α*-solanine has been shown to have an impact on the growth, development, and reproduction of insects.[27,28] Metabolomic analysis further revealed a significant accumulation of ephedrine in *B. bassiana*-treated tomatoes. Ephedrine is a well-known toxic substance.[32] Overall, our findings indicated that alkaloid synthesis was affected by *B. bassiana* colonization and suggested that *B.*

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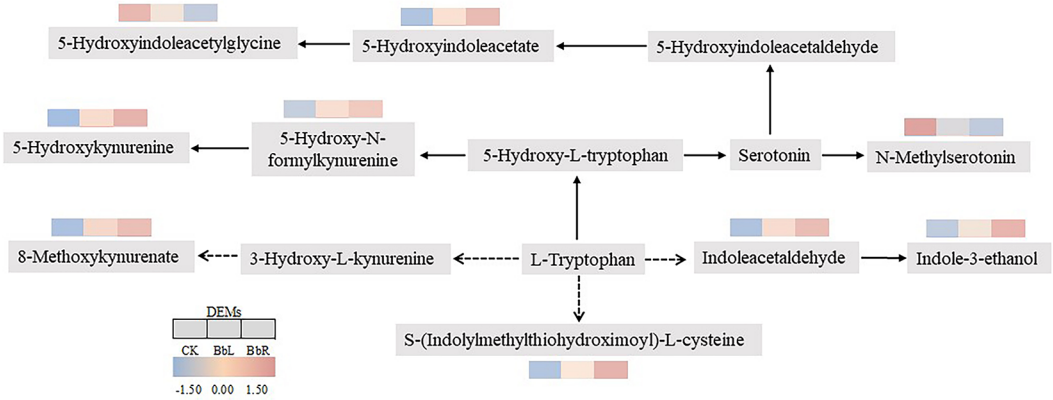

Figure 6. Differences in the metabolites involved in the tryptophan pathway were observed between tomato leaves colonized by *B. bassiana* and control leaves.

bassiana may inhibit whitefly occurrence by inducing the synthesis of SGAs in tomatoes.

B. bassiana colonization induces the synthesis of flavonoids in tomatoes. To depict the relationship in the flavonoid biosynthesis pathway, we constructed a network by integrating differential metabolites and genes (Figure 5). In comparison to the control, the application of *B. bassiana* through local leaf spraying and root irrigation resulted in upregulation of four flavonoid metabolites in tomatoes: phlorizin chalcone, 3,7,4′-tri-*O*-methylquercetin, leucocyanidin, and 8-*C*-glucosylnaringenin. Moreover, luteolin 7-*O*-*β*-D-glucoside and coumestrol exhibited a significant increase in tomatoes treated with local leaf spraying, while 5-*O*-caffeoylshikimic acid showed a notable increase in tomatoes treated with root irrigation. We also observed upregulation of several core genes involved in regulating the metabolites in tomatoes treated with root irrigation, including phenylalanine ammonia lyase (*PAL*), 4-coumarate-CoA ligase (*4CL*), *trans*-cinnamate 4-monooxygenase (*CYP73A*), and 5-*O*-(4-coumaroyl)-D-quinate 3′-monooxygenase (*CYP98A*). Flavonoids play a crucial role in plant defense against herbivores, because their presence can affect palatability, reduce nutritional value, decrease digestibility, or even function as toxins.[33,34] Our results suggested that the increase in flavonoid levels may contribute to the enhanced insect resistance in tomatoes induced by *B. bassiana*.

Our results revealed that colonization by *B. bassiana* had a stimulating effect on tryptophan metabolism and brassinosteroid biosynthesis (Figure 6 and Figure S3 of the Supporting Information). Specifically, tomatoes treated with *B. bassiana* through root irrigation exhibited the presence of nine differential metabolites. Among these, seven metabolites showed significant accumulation, including 8-methoxykynurenate, 5-hydroxyindoleacetate, indoleacetaldehyde, 5-hydroxykynurenine, *S*-(indolylmethylthiohydroximoyl)-L-cysteine, indole-3-ethanol, and 5-hydroxy-*N*-formylkynurenine. Conversely, two metabolites experienced a decrease, namely, 5-hydroxyindoleacetylglycine and *N*-methylserotonin. Brassinosteroids (BRs) act as steroid hormones that regulate plant growth and development. In our study, tomatoes treated with *B. bassiana* demonstrated a significant increase in brassinolide levels. Additionally, our analysis revealed that important genes involved in this process, such as plant 4,4-dimethylsterol *C*-4*α*-methyl-monooxygenase (*SMO1*), steroid 5-*α*-reductase (*DET2*), and brassinosteroid 6-oxygenase (*CYP85A1*), were significantly upregulated in the tomatoes treated with *B. bassiana* through root irrigation. We should note that secondary metabolites derived from tryptophan play a crucial role in plant development and defense responses.[35,36] These findings suggested that *B. bassiana* can enhance tomato resistance by activating tryptophan metabolism and brassinosterol synthesis.

α-Solanine Biosynthesis Genes Enhance the Resistance of Tomato to *B. tabaci*. In our study, we observed that the presence of *B. bassiana* led to an accumulation of *α*-solanine in the tomato plants. To further investigate, we conducted an experiment in which tomato leaves were sprayed with *α*-solanine and *B. tabaci* females were introduced 24 h later. The results indicated that the plants treated with *α*-solanine disrupted the feeding behavior in *B. tabaci*. Specifically, there was a significant decrease in the number of whiteflies and their oviposition compared to control plants (Figure S4 of the Supporting Information). Previous studies have identified four genes, *SGT1*, *SGT2*, *SGT3*, and *SGT4*, which regulate the synthesis of *α*-solanine through UDP-glucose:sterol glucosyltransferase (*SGT*). In our study, we focused on investigating the roles of *SlSGT2* and *SlSGT3*. We observed an upregulation in their expression with *B. bassiana* treatment through root irrigation, as confirmed by qRT-PCR analysis (Figure 7A). To elucidate the function of *SlSGT2* and *SlSGT3*, we used the TRV2 vector to silence these genes (Figure 7B) and achieved a silencing efficiency of over 60% (Figure 7C). Interestingly, silencing *SlSGT2* had a significant downregulating effect on *SlSGT3*, while silencing *SlSGT3* had no significant impact on *SlSGT2* (Figure 7C). Furthermore, we found that silencing of *SlSGT2* led to a significant reduction in the *α*-solanine content, whereas silencing *SlSGT3* had no significant effect (Figure 7D). We also examined the influence of gene silencing on the fecundity of female *B. tabaci* adults on the leaves of the treated tomatoes. Our results demonstrated that the silencing of *SlSGT2* and *SlSGT3* led to an increase in the fecundity of adults within 3 days (Figure 7E). Additionally, we monitored the number of nymphs on the leaves of treated tomatoes for a period of 20 days and observed a significantly higher number of nymphs on the leaves of *SlSGT2*-silenced tomatoes, while the silencing of *SlSGT3* had no significant effect (Figure 7F).

DISCUSSION

The interactions among plants, insects, and EPF in complex multitrophic systems have attracted significant interest in the

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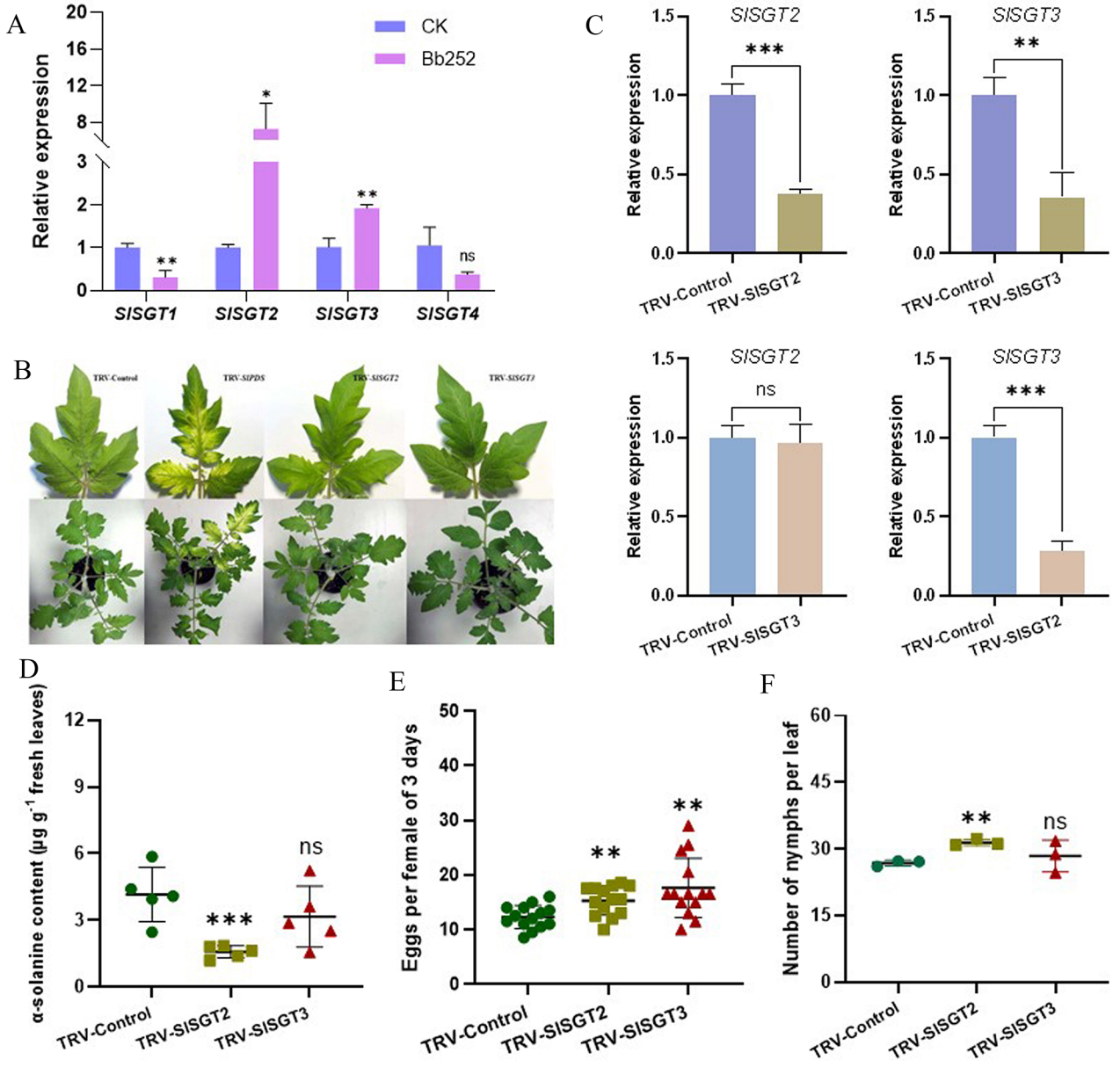

Figure 7. Silencing of α-solanine synthesis genes *SlSGT2* and *SlSGT3*: (A) quantitative analysis conducted on the gene responses for α-solanine biosynthesis, namely, *SlSGT1*, *SlSGT2*, *SlSGT3*, and *SlSGT4*, (B) silencing effect of the target gene, (C) detection of target gene silencing efficiency, (D) silencing of target genes inhibiting the production of α-solanine in tomato leaves, (E) silencing of the target gene increasing the fecundity of *B. tabaci* on tomato leaves within 3 days, and (F) silencing of the target gene increasing the number of nymphs on tomato leaves. All values are the mean ± standard deviation (SD), and asterisks indicate a significant difference compared to the control group using Student's *t* test. (∗, $p < 0.05$; ∗∗, $p < 0.01$; and ∗∗∗, $p < 0.001$).

field of biological control. Research has demonstrated that plant endophytic EPF have a substantial negative impact on herbivorous insects, especially those that rely on plant phloem as their food source.[37−39] In our study, we made a noteworthy observation that the fertility of whiteflies significantly decreased after the consumption of tomatoes treated with *B. bassiana*. Interestingly, we found that the effects were even more pronounced when *B. bassiana* was applied to the tomatoes through root irrigation rather than local leaf spraying. Both metabolome and transcriptome analyses provided further support for these outcomes, revealing numerous differential metabolites and genes in the tomatoes treated with *B. bassiana*. Differential metabolite analysis identified 16 metabolites with anti-insect activity, and the upregulation of the metabolome in the tomatoes treated by root irrigation was significantly higher compared to local leaf spraying. It is worth noting that metabolites, such as α-solanine, 5-*O*-caffeoylshikimic acid, and clerodendrin A, previously reported to inhibit the occurrence of pests,[29−31,40] were found to be significantly accumulated in the tomatoes treated with *B. bassiana* via root irrigation. These findings provided compelling evidence supporting the observed biological phenomena.

The analysis of multiple omics data is a valuable approach for understanding biological phenomena. In our study, we conducted a metabolome analysis to confirm the significant influence of the endophytic fungus *B. bassiana* on the accumulation of alkaloids, flavonoids, and tryptophan in tomato leaves. The transcriptional expression profiles of the

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plants also showed a consistent trend. Specifically, the toxic secondary metabolites known as specialized metabolites with antifeedant properties (SGAs) play a crucial role in nightshade crops when exposed to various biological stresses, such as pathogens and predators.[20,41−44] Previous studies have shown that *GAME9*, which is highly expressed in tomato tissues, serves as a central regulatory in SGA biosynthesis.[20,21,43] In our study, we discovered numerous genes involved in the regulation of SGA synthesis, such as *GAME9*, *GAME6*, and *GAME4*, in tomatoes treated with *B. bassiana* via root irrigation. Additionally, we observed a significant increase in the level of SGA α-solanine in tomatoes treated with *B. bassiana* through root irrigation. Thus, we hypothesized that *B. bassiana* enhances tomato resistance to whiteflies by modulating SGA synthesis, particularly α-solanine. Furthermore, plant flavonoids have been shown to regulate insect-feeding and egg-laying behavior.[45] In our study, we identified several upregulated flavonoid-related metabolites and observed a significant upregulation of genes involved in flavonoid synthesis, including 3,7,4′-tri-*O*-methylquercetin, leucocyanidin, 8-*C*-glucosylnaringenin, and 5-*O*-caffeoylshikimic acid, along with their corresponding synthetic genes *FLS*, *CYP75A*, *DFR*, *HCT*, and *CYP98A*, respectively, in tomatoes treated with *B. bassiana* through root irrigation. Notably, 5-*O*-caffeoylshikimic acid has been shown to inhibit the spawning of adult worms in *Schistosoma mansoni*,[40] suggesting that endophytic *B. bassiana* may activate the flavonoid biosynthetic pathway to enhance tomato resistance to insects. Tryptophan, a precursor of auxin and various secondary metabolites in plants, plays a vital role in protecting plants against fungal, bacterial, and herbivore attacks.[46,47] Elevated levels of tryptophan-derived indoles have been associated with an increase in salicylic acid (SA).[48] Our results demonstrated a significant accumulation of seven tryptophan-derived metabolites, namely 8-methoxykynurenate, 5-hydroxyindoleacetate, indoleacetaldehyde, 5-hydroxykynurenine, *S*-(indolylmethylthiohydroximoyl)-L-cysteine, indole-3-ethanol, and 5-hydroxy-*N*-formylkynurenine, in tomatoes treated with *B. bassiana* via root irrigation. These findings suggested that *B. bassiana* can enhance systemic resistance in tomatoes by activating the tryptophan metabolism. Moreover, this provided a strategy to induce plant defense responses through the application of tryptophan. Overall, our study convincingly demonstrated that endophytic *B. bassiana* significantly impacted the accumulation of metabolites involved in plant defense mechanisms.

The analysis of metabolome and transcriptome data has provided valuable insights into the impact of local leaf spraying and root irrigation on tomato leaves. The observed variations in metabolite components and gene expression patterns suggested that root irrigation with *B. bassiana* had a more substantial effect. Given the crucial role of soil microorganisms in terrestrial biogeochemistry and their metabolic functions influenced by ecological interactions with other soil microbial populations, soil fauna and plants, and the surrounding soil environment,[49−53] it is imperative to investigate the relationship between soil microorganisms and the observed effects on tomatoes treated with *B. bassiana* via root irrigation. This investigation is of great importance and warrants further exploration and in-depth analysis to enhance our understanding of the complex interactions among plants, microorganisms, and the soil environment.

In conclusion, our study investigates the metabolomic and transcriptomic analyses of tomato plants infected with *B. bassiana* against whiteflies, providing valuable insights into the mechanisms underlying enhanced plant resistance. The activation of metabolic pathways, including alkaloids, flavonoids, and tryptophan, in tomato plants indicates their involvement in defense responses against whiteflies. Additionally, our findings demonstrate that gene silencing of *SGT2* can suppress whitefly reproduction by regulating the synthesis of α-solanine. This discovery highlights the potential use of *B. bassiana* as a biological control agent for managing whiteflies in sustainable agriculture practices. The uniqueness of our study lies in the "whole system approach" that we employed, enabling us to comprehensively analyze the mechanism by which *B. bassiana* enhances tomato resistance. This approach provides a more holistic understanding of the interactions among *B. bassiana*, tomato plants and whiteflies. Overall, our research contributes to a better comprehension of the multitrophic interactions involved in enhancing plant resistance and offers valuable insights for future agricultural practices.

■ ASSOCIATED CONTENT

Data Availability Statement

The raw RNA sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in the National Genomics Data Center (Nucleic Acids Research 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences [Genome Sequence Archive (GSA) CRA010678] that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c03679.

Differential metabolite accumulations observed in tomatoes treated with *B. bassiana* compared to the control group (Figure S1), quantitative PCR analysis conducted on SGA synthesis genes (Figure S2), changes of brassinosteroid biosynthesis metabolites and genes in tomato leaves colonized by *B. bassiana* as well as in control tomato leaves (Figure S3), and exogenous α-solanine reducing the fitness of *B. tabaci* (Figure S4) (PDF)

Primers used for analysis (Table S1), metabolic changes of tomato leaves between the *B. bassiana* colonization of leaf spraying (BbL) and the control (CK) group (Table S2), metabolic changes of tomato leaves between the *B. bassiana* colonization of root irrigation (BbR) and the control (CK) group (Table S3), RNA-Seq statistical summary (Table S4), transcriptomic changes of tomato leaves between the *B. bassiana* colonization of leaf spraying (BbL) and the control (CK) group (Table S5), and transcriptomic changes of tomato leaves between the *B. bassiana* colonization of root irrigation (BbR) and the control (CK) group (Table S6) (XLSX)

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Author Contributions

Xian Wang, Ziying Wang, and Huai Liu conceived and designed the experiments. Xian Wang, Wenjie Liu, Haolin Chen, Ganwei Yan, and Qian Yuan conducted the experiments. Xian Wang, Wenjie Liu, and Ganwei Yan analyzed the data. Xian Wang and Ziying Wang wrote the manuscript. All authors read and approved the manuscript.

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Notes

The authors declare no competing financial interest.

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