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REGULAR ARTICLE

Pseudomonas koreensis promotes tomato growth and shows potential to induce stress tolerance via auxin and polyphenol-related pathways

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Abstract

Aims *Pseudomonas koreensis*, a subgroup of the *P. fluorescens* complex, is a potential plant growth-promoting rhizobacterium. This study explored the mechanisms of plant growth promotion by *P. koreensis* and its potential to induce stress tolerance in tomato.

Methods Tomato plants in pots were inoculated with *P. koreensis* GS and cultured for 60 days. RNA sequencing and gas chromatography-mass spectrometry were used to detect global transcriptomic and metabolomic changes in tomato leaves. Liquid chromatography-mass spectrometry was used for quantification of plant hormones.

Results The inoculated plants showed more vigorous growth, with higher leaf chlorophyll content and shoot biomass compared with uninoculated controls. The activities of several defense enzymes (e.g., phenylalanine ammonia-lyase) were enhanced in the leaves of inoculated plants. A total of 737 differentially expressed genes were identified, which were related to plant hormone biosynthesis, MAPK signaling transduction, and polyphenol biosynthesis. The contents of specific metabolites related to plant growth and stress tolerance, including polyphenols (phenylpropanoids) and amino acids (tryptophanand and proline), were increased after inoculation. Plant hormones such as indole-3-acetic acid participated in plant growth promotion by *P. koreensis*.

Conclusions *P. koreensis* promoted plant growth and showed potential to induce stress tolerance in tomato by enhancing auxin and polyphenol-related pathways.

Keywords *Pseudomonas koreensis* · Growth promotion · Stress tolerance · Polyphenols · Transcriptome · Metabolome

Abbreviations

ABA	Abscisic acid
BAK1	Brassinosteroid insensitive 1-associated receptor kinase 1
DEGs	Differentially expressed gene
GA	Gibberellic acid
GID2	GA-insensitive dwarf 2
JA	Jasmonic acid
PAL	Phenylalanine ammonia-lyase
PIF3	Phytochrome-interacting factor 3
PPO	Polyphenol oxidase
RNA-Seq	RNA sequencing
TCH4	xyloglucan:xyloglucosyl transferase TCH4
WRKY33	WRKY transcription factor 33
Aux/IAA	Auxin-responsive protein/IAA
CAT	Catalase
FLS2	Flagellin-sensing 2
GH3	Gretchen Hagen 3
IAA	Indole-3-acetic acid

Q. Guo and Y. Sun contributed equally to this work.

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KEGG	Kyoto Encyclopedia of Genes and Genomes
PGPR	Plant growth-promoting rhizobacterium
POD	Peroxidase
PR1	Pathogenesis-related protein1
SA	Salicylic acid
VIP1	VirE2-interacting protein 1

Introduction

China is the world's leading producer of tomato, accounting for approximately 20.9 % (999,312 ha) of global harvested area and 31.8 % (5.631×10^{10} kg) of global production in 2016. However, the average yield per hectare of tomato in China (56,350 kg) is still lower than the global average (Wang et al. 2018). At least 10 diseases cause significant tomato yield reduction in China (Li 2011). In recent years, the application of beneficial microbes as biocontrol agents has received much attention because they are environmentally friendly, low in cost, and easy to use.

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that benefit plants by improving plant productivity and immunity (Porcel et al. 2014). *Pseudomonas*, *Bacillus*, and *Streptomyces* spp. are the commonest PGPR, extensively used for plant growth promotion and disease control (Fahimi et al. 2014; Li et al. 2019; Lim and Kim 2009). Widely accepted mechanisms for plant growth promotion by PGPR include: (1) modulation of the biosynthesis of plant growth regulators such as indole-3-acetic acid (IAA) (Acuna et al. 2011) and gibberellic acid (GA) (Ahmed et al. 2014); (2) enhancement of the solubilization of mineral phosphates and other nutrients with low bioavailability in soil (Hayat et al. 2010); and (3) direct participation in atmospheric nitrogen fixation or indirect contribution to nitrogen fixation efficiency of rhizobia (Figueiredo et al. 2008; Habibi et al. 2014).

Evidence suggests that PGPR can control plant diseases through direct mechanisms—antagonistic activity against pathogens via competition for nutrients and production of antibiotics or lytic enzymes, and indirect mechanisms—induction of plant resistance to pathogens and regulation of mitogen-activated protein kinase (MAPK) cascades in plants (Pieterse et al. 2014). When plants suffer abiotic stresses such as drought, salinity, and heavy metals, PGPR can be involved in the upregulation of plant secondary metabolic pathways such as polyphenol synthesis (Kumar et al. 2016). Polyphenols including flavonoids, phenylpropanoids, anthocyanins, and lignins can help plants withstand various biotic and abiotic stresses (Martins et al. 2013; Moura et al. 2010; Trinh et al. 2018; Wang et al. 2016).

Pseudomonas represents a major group of PGPR and members such as *P. fluorescens* have been reported to be beneficial microbes that mediate plant growth promotion and/or disease suppression (Lopes et al. 2018). *P. koreensis* is a subgroup of closely related strains within the *P. fluorescens* complex (Rangel et al. 2016; Rafikova et al. 2016) reported that in a field test that *P. koreensis* strain IB-4 had a positive effect on potato yield and resistance to plant pathogens through its antifungal activity, nitrogenase activity, and ability to synthesize IAA and cytokinin-like compounds. Hultberg et al. (2010) showed that a biosurfactant produced by *P. koreensis* strain 2.74 suppressed disease in tomato plants infected by *Pythium ultimum* in greenhouse trials. These studies suggest that *P. koreensis* is a potential PGPR species. Our recent study showed that the addition of cell-free filtrate from *P. koreensis* strain GS promoted the growth of tomato seedlings under laboratory conditions (Guo et al. 2020). However, there is a lack of systematic research to clarify the mechanisms of plant growth promotion by strain GS and the global transcriptomic and metabolomic changes in plants elicited by *P. koreensis*.

In the present study, we investigated the effects of *P. koreensis* GS on plant growth and defense enzyme activity in tomato. RNA sequencing (RNA-Seq) and metabolome analysis were used to explore the possible mechanisms underpinning plant growth promotion by *P. koreensis* and its potential to induce stress tolerance in plants. We hypothesized that: (1) *P. koreensis* promotes the growth of tomato plants by increasing the expression of functional genes and specific metabolites related to plant growth; and (2) *P. koreensis* shows potential to induce stress tolerance in tomato plants via enhancing defense enzyme activities and the biosynthesis of defense metabolites such as polyphenols and amino acids. This study presents the first evidence of transcriptomic and metabolic changes that occur in tomato plants treated with *P. koreensis*.

Materials and methods

Strain, seeds, and soil

Pseudomonas koreensis strain GS (PRJNA517377) was previously isolated from the rhizosphere of ginseng

(*Panax ginseng* C.A. Mey) in a brown loam soil in the southern piedmont of the Lesser Khingan Mountains, China (46°57' N, 128°16' E). Strain GS was preserved by the Resource and Environmental Biology Laboratory in the College of Resources & Environment, Northwest A&F University (Yangling, Shaanxi, China). Seeds of tomato (*Lycopersicon esculentum* Mill.) cultivar 'Jinpeng 1' were purchased from Xi'an Jinpeng Seedling Co., Ltd. (Xi'an, Shaanxi Province, China). Fertile field soil was collected from farmland in Yangling. The soil type was classified as Eum-Orthric Anthrosols (Staff 2010). The soil had a pH of 7.67 and contained total organic carbon of 12 g kg^{-1}, total nitrogen of 1.66 g kg^{-1}, available phosphorous of 26.3 mg kg^{-1}, and available potassium of 155.9 mg kg^{-1}. The soil was passed through a 2.0-mm sieve and mixed thoroughly with compound fertilizer (N:P:K, 15:6.5:12.4) at a rate of 3 ‰ before use.

Pot experiments

Tomato seeds (three seeds per pot) were drilled into 60 pots (diameter × height, 15 × 12 cm) each containing 1.2 kg of fresh soil. All pots were placed in a greenhouse at 28 °C, with sunlight for 12 hours per day on average. The pots were watered once a day with 120 mL of sterilized distilled water per pot. When the first true leaves appeared, one seedling with uniform growth was selected and retained in each pot. The experimental design was a randomized complete block design with three blocks, and each block contained 20 pots. The pots in each block were divided randomly and equally into a control group and treatment group, with 10 plants per group.

Strain GS was cultured in beef extract peptone broth at 37 °C with oscillation at 180 rpm. The 72-hour-old culture was diluted with sterile phosphate-buffered saline. After the first true leaves had just emerged, one group of tomato seedlings was watered with 100 mL of diluted cell culture (1×10^7 mL^{-1} colony-forming units) per pot (treatment group), and the other group received 100 mL of sterile phosphate-buffered saline per pot (control group). No pesticides or fungicides were applied, and the plants did not show any symptoms of disease during the experiment.

Plant growth measurements and leaf sampling

Plant height and stem diameter were measured 15, 30, 45, and 60 days post-inoculation (dpi). Leaf chlorophyll content (SPAD value) was measured at 30 and 60 dpi using a SPAD-502plus (Konica Minolta, Tokyo, Japan). Fresh weight of shoot biomass was measured at 60 dpi.

Leaf samples were taken at 30 dpi for physiological and molecular analyses. For enzyme activity assays, three biological samples (replicates) were taken from each group, and a composite sample of biological replicates was obtained from a mixture of the 4th and 5th leaves five of plants randomly collected in each block. For transcriptome analysis and quantitative validation of functional gene expression, three biological samples (replicates) were taken from each group, and a composite sample of biological replicates was obtained from a mixture of the 3rd leaf of five plants randomly collected in each block. For metabolome and hormone analyses, seven biological samples (replicates) were taken from each group, and each biological replicate was obtained from the 2nd leaf of one plant randomly selected in three blocks. Leaf samples used for enzyme activity assays were directly placed into an ice-box after collection. The remaining leaf samples were washed with distilled water and dried on blotting paper, then immediately frozen in liquid nitrogen. All samples were taken to the laboratory and stored at − 80 °C until use.

Defense enzyme activity assays

Polyphenol oxidase (PPO) activity was assayed using the method of Li (1998). An absorbance change of 0.001 units per min at 398 nm was defined as one unit of PPO activity (unit: U min^{-1}). Catalase (CAT) activity was assayed using the method of Gao (2006). An absorbance change of 0.01 units per min at 240 nm was defined as one unit of CAT activity (unit: U min^{-1}). Phenylalanine ammonia-lyase (PAL) and peroxidase (POD) activities were assayed using the method of Silva et al. (2004). An absorbance change of 0.01 units per min at 290 nm (or 470 nm) was defined as one unit of PAL (or POD) activity (unit: U min^{-1}).

Transcriptome analysis

RNA isolation and sequencing

Leaf samples were sent to BGI (Shenzhen, China) for RNA isolation and transcriptome sequencing. Total RNA was isolated from leaf samples (each 0.1 g) using an RNeasy mini kit (Qiagen, Hilden, Germany). RNA purity was determined using a NanoPhotometer® (IMPLEN, Westlake Village, USA). RNA

concentration was measured using a Qubit® RNA Assay Kit in a Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, USA). The RNA integrity number (RIN) was assessed using an RNA Nano 6000 Assay Kit on the Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, USA). The samples were considered acceptable if $2.2 \geq A_{260}/A_{280} \geq 2.0$, 28 S/JZ18 S ≥ 1.5, and RIN ≥ 7.5.

Library preparations were sequenced on the BGISEQ-500 platform (BGI, Shenzhen, China), and 50-bp single-end reads were generated. Raw reads were first processed using SOAPnuke (parameters: −l 5 − q 0.5 − n 0.1) and then filtered using Trimmomatic (parameters: ILLUMINACLIP:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:50). The obtained clean reads were aligned to the tomato reference genome using HISAT and then mapped to the reference sequence using Bowtie2 (Johns Hopkins University, Baltimore, MD, USA). The gene and transcript expression levels of each sample were calculated using RSEM (http://deweylab.biostat.wisc.edu/rsem/).

The open reading frame (ORF) of each unigene was detected using getorf (http://emboss.sourceforge.net/apps/cvs/emboss/apps/getorf.html). The ORFs were aligned to transcription factor domains from the Plant Transcription Factor database (PlnTFDB; http://plntfdb.bio.uni-potsdam.de/) using hmmsearch (http://hmmer.org/). The function of unigenes was identified based on the characteristics of transcription factor families described by PlnTFDB (http://plntfdb.bio.uni-potsdam.de/v3.0/).

Identification of differentially expressed genes (DEGs)

Differential gene expression analysis was performed using the method of Zhao et al. (2018). High-quality clean reads were mapped to the reference transcriptome, and the transcript levels of unigenes were analyzed using TopHat (http://ccb.jhu.edu/software/tophat/index.shtml) and Cufflinks (http://cole-trapnelllab.github.io/cufflinks/). The transcript levels were normalized to Fragments Per Kilobase of exon model per Million mapped reads (FPKM) (Trapnell et al. 2010) as follows: FPKM = 106 *C*/*NL*/103, where *C* denotes the number of fragments aligned to a particular gene, *N* denotes the total number of fragments aligned to all genes, and *L* denotes the number of bases in the particular gene. Sequencing was used to identify genes that were differentially expressed in different groups. False discovery rates (FDRs) were used in multiple hypothesis testing to correct p-values. The FDRs were statistically preset to values < 0.05, and differential gene expression was calculated based on FPKM values (Zenoni et al. 2010). An absolute value of |fold-change| > 2 and $p < 0.001$ were used as the thresholds to screen DEGs.

Gene annotation and functional enrichment analysis

Gene Ontology (GO) analysis of DEGs was performed using the PANTHER (Protein Annotation Through Evolutionary Relationship) classification system (http://www.pantherdb.org/data/). Pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.kegg.jp/). The Basic Local Alignment and Search Tool (BLASTn) was used to identify homologues in the tomato genome (http://solgenomics.net/).

Validation of transcriptome data by real-time PCR

Reverse transcription quantitative real-time PCR (RT-qPCR) was used to validate the RNA-Seq data for selected DEGs. The RNA used for quantitative validation of functional gene expression was derived from RNA extracted for transcriptome analysis, and random primers (Table S1) were used for reverse transcription. Total RNA (500 ng) was reverse-transcribed into cDNA using the following program: 25 °C for 10 min, 42 °C for 20 min, 85 °C for 5 s, and holding at 12 °C. Then, 1 μL of cDNA was used as template for RT-qPCR in the following conditions: 95 °C for 180 s, followed by 40 cycles of 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s. Data acquisition and analysis were performed using Bio-Rad iQ5 software (V.2.1.97; Bio-Rad Laboratories, Hercules, USA), with the number of cycles required for the fluorescent signal to cross the threshold in RT-qPCR as the cycle threshold (CT) (Ali-Benali et al. 2005). Actin served as an internal reference. The relative expression level of target genes was calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak 2008).

Metabolome and hormone analyses

Gas chromatography-mass spectrometry (GC-MS) of metabolites

Leaf samples (each 100 mg) and five steel beads were transferred into 5-mL centrifuge tubes. Following

pretreatment with liquid nitrogen (5 min), the tubes were placed in a high-flux tissue grinding apparatus (SCIENTZ-48; Xinzhi, Ningbo, China) at 70 Hz for 1 min. Then, 400 μL of methanol (precooled to −20 °C) were added to each tube, followed by the addition of 60 μL of ribitol (0.2 mg mL^{-1} stock in methanol) as an internal quantitative standard. After vortexing, the mixtures were sonicated at room temperature for 30 min. Then, 750 μL of chloroform (precooled to −20 °C) and 1,400 μL of deionized water (4 °C) were added to each tube. The mixtures were vortexed for 60 s and then centrifuged at 18,756 x g for 10 min at 4 °C.

Aliquots (each 1 mL) of the supernatants were transferred into fresh centrifuge tubes and dried by vacuum concentration. Afterward, 60 μL of methoxyamine pyridine solution (15 mg mL^{-1}) was added to each tube. The mixtures were vortexed for 30 s and allowed to react for 120 min at 37 °C. Then, 60 μL of N, O-*bis* (trimethylsilyl) trifluoroacetamide reagent (containing 1 % trimethyl chlorosilane) was added and the mixtures were allowed to react for another 90 min at 37 °C. Following reaction, the tubes were centrifuged at 13,780 x g for 10 min at 4 °C and the supernatants were collected for use. For quality control, 20 μL of each prepared sample was pooled to determine deviations of the analytical results, and the deviations from pooled mixtures were then compared to analytical errors. The remaining samples were used for GC-MS analysis.

To separate the derivatives, GC was performed on an HP-5MS capillary column (30 m × 250 μm i.d., 0.25 μm film thickness; Agilent J 7980A; Agilent J & W Scientific, Folsom, USA) coated with 5 % phenyl and 95 % methylpolysiloxane. Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. Sample (each 1 μL) was injected in split mode with a 20:1 split ratio using an autosampler. The injection temperature was maintained at 280 °C, with the interface set to 150 °C and the ion source adjusted to 230 °C. The temperature ramping program was as follows: start at 60 °C for 2 min, ramp to 300 °C at 10 °C min^{-1}, hold at 300 °C for 5 min. MS was performed using the full-scan mode over the mass-to-charge ratio range from *m/z* 35 to 750 (Agilent J 5975C; Agilent J & W Scientific, Folsom, USA).

To classify and annotate metabolites, the raw data were converted into netCDF format using G1701 MSD ChemStation (E.02.00.493) and then preprocessed using the XCMS package of R (v3.3.2) for peaks identification, peaks filtration, and peaks alignment. The main parameters were as follows: bw = 2, minfrac = 0.3, fwhm = 3, snthresh = 0, step = 0.1, steps = 2, mzdiff = 0.5, and profmethod = binlin. The data matrix including the mass-to-charge ratio (*m*/*z*), retention time (RT), and peak area (intensity) was obtained. After that, annotation of the metabolites was performed using the AMDIS program combined with the National Institute of Standards and Technology (NIST) commercial database (http://www.nist.gov/) and Wiley registry metabolome database (http://sciencesolutions.wiley.com/.). Among them, the metabolite alkane retention index was used for further substance identification according to the retention index provided by The Golm Metabolome Database (GMD; http://gmd.mpimp-golm.mpg.de). To compare the data of different magnitudes, the peak area of the data was normalized by the internal standard. Finally, the metabolites obtained by annotation were divided into nine categories, amino acids, sugars, nucleotides, organic acids, fatty acids, phosphoric acids, amines, polyols, and others. The screening conditions of differential metabolites were as follows: variable importance in the project (VIP) ≥ 1 and p-value ≤ 0.05.

Liquid chromatography-mass spectrometry (LC-MS) of plant hormones

Leaf samples (each 0.3 g) were extracted with 10 mL of acetone–methanol solution (4:1, v/v) by shaking for 1 min and incubating in a 55 °C water bath for 1 h. After centrifugation (13,780 x g, 4 °C), the supernatants were collected into fresh tubes with 5 mL of acetone–methanol solution, followed by shaking for 1 min and incubation at 55 °C for 30 min. After centrifugation (13,780 x g, 4 °C) and concentration, the materials were redissolved with 240 μL of 80 % methanol and filtered through a 0.22-μm microporous filter. The filtrates were analyzed by LC-MS. The samples were protected from light and kept at low temperature during preparation and analysis.

LC was conducted using an ACQU ITY UPLC® BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters, Milford, USA). The injection volume was 5 μL and the column temperature was maintained at 40 °C. The mobile phase consisted of 0.1 % acetic acid (A) and acetonitrile (B). The gradient elution conditions were: 0–2 min, 90–75 % A; 2–3 min, 75 % A; 3–4 min, 75–10 % A; 4–5 min, 10 % A; 5–6 min, 10–90 % A; and 6–8 min, 90 % A. MS was conducted using an electrospray ionization source in both positive and negative ionization modes, with an ion source temperature of 500 °C

and ion source voltage of 5000 V/−4500 V. Collision gas was maintained at 6 psi, with air curtain gas at 30 psi, and atomization and auxiliary gases at 50 psi. Multipath monitoring was used for scanning (Bajad et al. 2006). The linearity of the measurement was evaluated by analyzing different concentrations of standard solutions of IAA, abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA). Calibration standards were prepared by diluting stock solutions to give the concentrations indicated in Table S2.

Statistical analysis

Data are expressed as means ± standard deviation (SD). The comparison of group means was performed using Student's *t*-test with DPS v4.0 (Data Processing System; http://www.chinadps.net). A *P*-value of < 0.05 was considered to indicate a significant difference. Enrichment of DEGs in biotic stress pathways was analyzed using MAMPAN (Thimm et al. 2004). Hierarchical cluster of differential metabolites was constructed by the pheatmap package in R v3.3.2 (http://www.r-project.org/). The correlations between all DEGs (fold-change data) and differential metabolites (normalized peak area data) involved in the same metabolic pathway were determined using the package psych in R (http://personality-project.org/r/psych) based on metabolic pathways recalculated at the KEGG website (http://www.kegg.jp/). Pearson's correlation coefficient was used to evaluate the consistency between RNA-Seq and RT-qPCR data.

Results

Plant growth promotion by *P. koreensis* GS

After treatment with *P. koreensis* GS, the growth and physiological parameters of tomato plants generally increased compared with those in the control group (Fig. 1). Plant height (Fig. 1b) and stem diameter (Fig. 1c) in the treatment group were respectively 6.6–19.5 % and 14.6–22.3 % higher than those in the control group from 30 to 60 dpi. Leaves from the treated plants appeared to be greener and their SPAD values increased significantly, by 15.1 % ($P < 0.05$; 30 dpi) and 8.3 % ($P < 0.05$; 60 dpi), compared with values for the control group (Fig. 1d). Significant increases ($P < 0.05$) also occurred in fresh weight of the treatment group at 60 dpi (61.5 %; $P < 0.05$) compared with the control group (Fig. 1e). The growth-promoting effect of *P. koreensis* GS was verified in terms of plant dry weight in an independent experiment (Note S1; Fig. S1).

Enhancement of defense enzyme activities by *P. koreensis* GS

Several defense enzymes showed higher activities in tomato leaves from the treatment group than in those from the control group (Fig. 2). Leaf PPO (Fig. 2a), CAT (Fig. 2b), and PAL (Fig. 2c) activities in the treatment group increased at 30 and 60 dpi, by 18.8–27.3 %, 38.8–25.3 %, and 11.3–22.4 % respectively, compared with levels in the control group. With regard to leaf POD activity, the treatment group showed a slight, but not significant, decrease at 30 dpi compared with the control group, whereas a 39.8 % increase occurred at 60 dpi (Fig. 2d).

Global transcriptomic changes induced by *P. koreensis* GS

Overall changes in gene expression

A total of 23,252 transcripts, amounting to ~ 68 % of the reference transcripts ($n = 33{,}810$), were detected in tomato leaves. From these, 737 genes (3.2 %) were identified as DEGs; among them, 571 were upregulated and 166 were downregulated in plants treated with strain GS relative to the control group. Although most of the DEGs have been functionally annotated, 35 (4.7 %) remain of unknown function, and 22 (3.0 %) encode either hypothetical proteins or proteins with putative functions. The transcriptome data were deposited in the Sequence Read Archive with accession number PRJNA544688.

GO functional enrichment analysis of DEGs

Functional enrichment analysis revealed that the 737 DEGs were enriched in 1407 GO terms (Table S3). Significant expression differences were found in 196 GO terms in the categories biological processes (110, 56.1 %), cellular components (24, 12.2 %), and molecular functions (62, 31.6 %). In biological processes, 51, 46, 41, 41, 33, 29, and 28 DEGs were associated with catabolic process, cellular catabolic process, carbohydrate metabolic process, organic

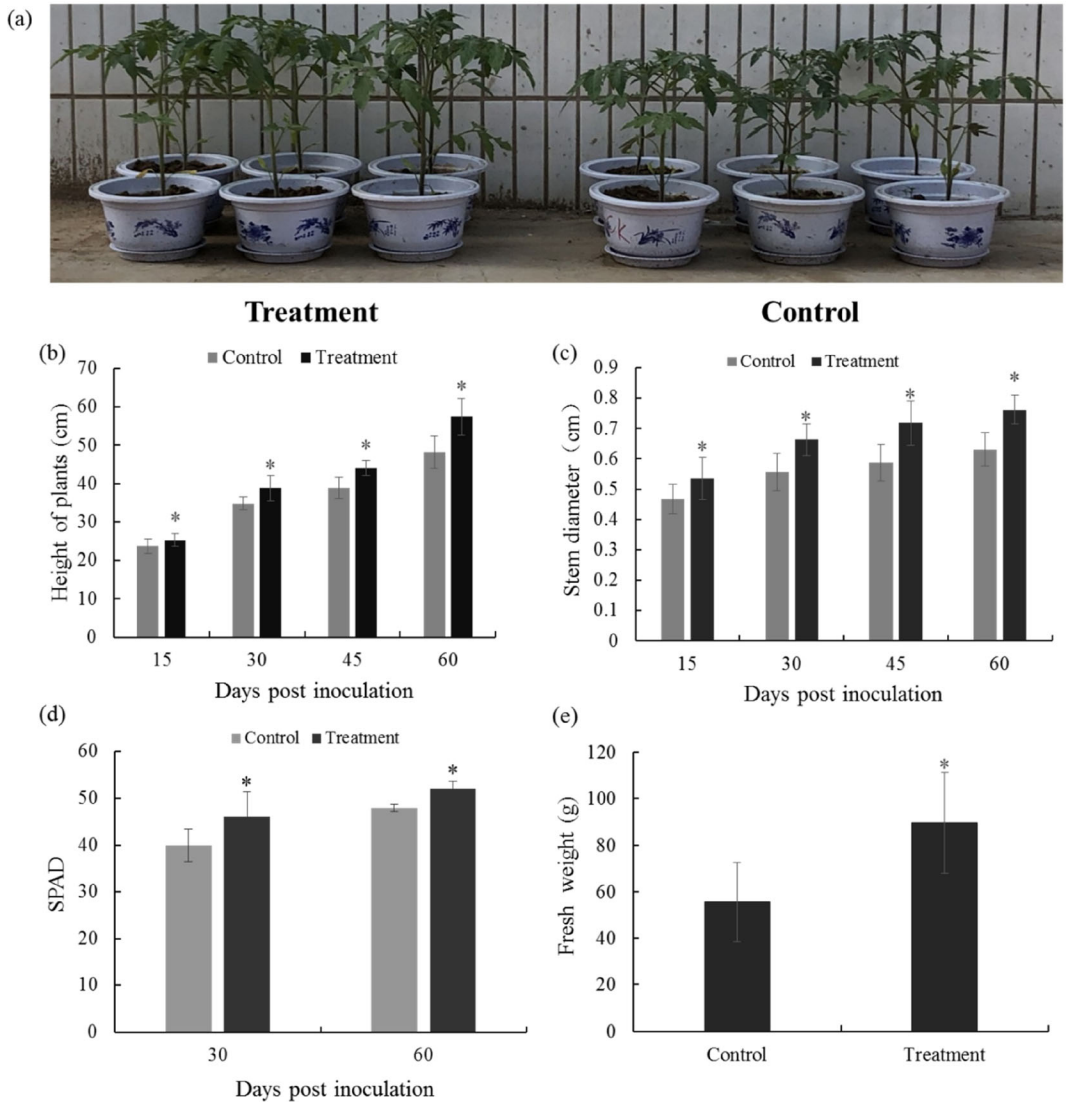

Fig. 1 Plant growth of tomato in the presence and absence of *P. koreensis* GS. (**a**) A photograph of potted plants 60 days post-inoculation; (**b**) plant height; (**c**) stem diameter; (**d**) leaf chlorophyll content (SPAD value); and (**e**) shoot biomass (fresh weight). Data are the means of 30 biological replicates (3 × 10). * $P < 0.05$ compared with control group (Student's t-test)

substance catabolic process, cell wall organization or biogenesis, lipid metabolic process, and secondary metabolic process, respectively. In cellular components, 194 and 178 DEGs were related to membrane and membrane part, respectively. In molecular functions, 116 and 116 DEGs were related to metal ion binding and cation binding, respectively.

KEGG pathway enrichment analysis of DEGs

The 737 DEGs were mapped to the KEGG database and 95 enriched pathways were identified. Table 1 lists the top 11 significantly enriched pathways, namely flavonoid biosynthesis, photosynthesis-antenna proteins, phenylpropanoid biosynthesis, flavone and flavonol biosynthesis, stilbenoid, diarylheptanoid and gingerol biosynthesis, anthocyanin biosynthesis, brassinosteroid biosynthesis, and MAPK signaling pathway-plant. Unigenes assigned to these pathways are summarized in Table S4.

Differential gene expression in functional pathways

Growth regulation KEGG analysis revealed that two DEGs involved in GA signal transduction were

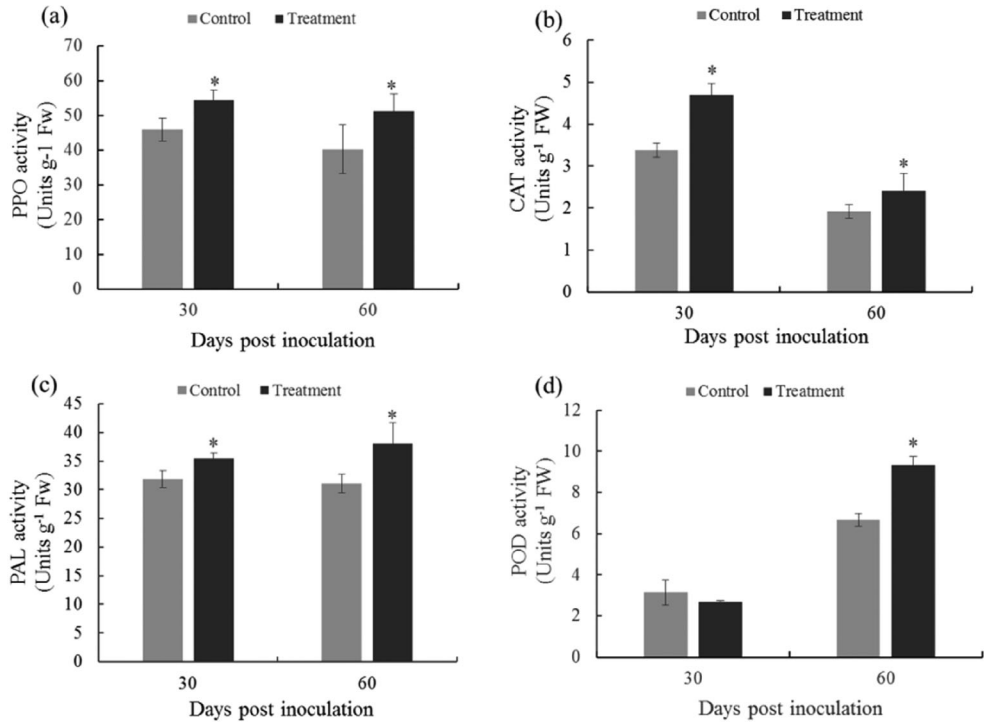

Fig. 2 Defense enzyme activities in the leaves of tomato plants inoculated with and without *P. koreensis* GS. (**a**) Polyphenol oxidase (PPO) activity; (**b**) catalase (CAT) activity; (**c**) phenylalanine ammonia-lyase (PAL) activity; and (**d**) peroxidase (POD) activity. Data are the means of three biological replicates. *$P < 0.05$ compared with the control group (Student's t-test)

upregulated in the treatment group compared with the control group (Fig. 3). GA receptor genes *GID2* (GA-insensitive dwarf 2) and *PIF3* (phytochrome-interacting factor 3) were upregulated by 5.2- and 2.2-fold, respectively. In auxin signal transduction, one early auxin response genes *Aux/IAA* (auxin-responsive protein/IAA) and two *GH3* (Gretchen Hagen 3) were upregulated by 2.2-, 2.5-, and 2.9-fold, respectively, while *SAUR* (small auxin-up RNA) was downregulated by 20.8-fold. All five DEGs associated with brassinosteroid biosynthesis were upregulated. Specifically, *BAK1* (brassinosteroid insensitive 1-associated

Table 1 The 11 most enriched KEGG pathways of differentially expressed genes (DEGs) identified in tomato leaves after treatment with *P. koreensis* GS

KEGG pathway	DEG number	Background gene number	p-value	Unigenes
Flavonoid biosynthesis	21	180	1.60E-08	Table S3
Photosynthesis–antenna proteins	8	31	1.18E-06	
Phenylpropanoid biosynthesis	32	585	1.11E-04	
Flavone and flavonol biosynthesis	5	21	1.98E-04	
Stilbenoid, diarylheptanoid and gingerol biosynthesis	10	127	2.29E-03	
Anthocyanin biosynthesis	3	13	4.55E-03	
Brassinosteroid biosynthesis	4	29	7.26E-03	
MAPK signaling pathway-plant	34	847	1.28E-02	
Monoterpenoid biosynthesis	3	20	1.58E-02	
Circadian rhythm-plant	12	229	2.16E-02	
Lipoic acid metabolism	2	11	3.40E-02	

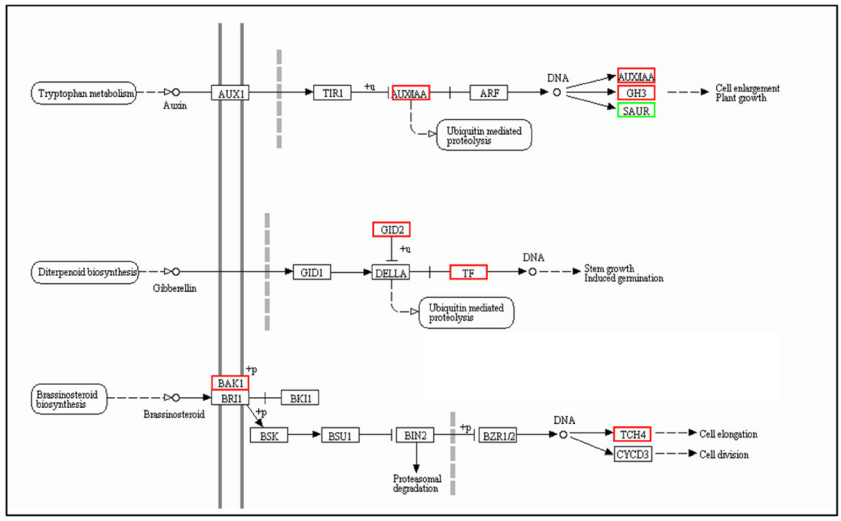

Fig. 3 Regulation of plant hormone biosynthesis and response pathways in tomato leaves by *P. koreensis* GS. The red box represents upregulation and the green box represents downregulation. Hormone signal transduction: KEGG PATHWAY Map04075 (http://www.genome.jp/dbget-bin/www_bget?map04075). (For interpretation of color in this figure, please refer to the online version of this article)

receptor kinase 1) was upregulated by 4.0-fold, and four *TCH4* (xyloglucan: xyloglucosyl transferase) genes were upregulated by 2.0- to 3.5-fold.

MAPK signaling pathway-plant KEGG analysis showed that there were 34 DEGs involved in the pathway MAPK signaling pathway-plant, 27 (79.4 %) of which were upregulated. Six DEGs were identified as leucine-rich repeat receptor-like serine/threonine-protein kinase genes, *FLS2* (flagellin-sensing 2), four of which were upregulated by 2.2–26.8-fold. In addition, the leucine-rich repeat receptor-like serine/threonine-protein kinase gene *ER* (ERECTA) was upregulated by 4.1-fold.

A number of genes downstream of the MAPK pathway were also upregulated by 2.1–3.5-fold, including *MKK4_5* (mitogen-activated protein kinase kinase 4/5), *MKS1* (MAP kinase substrate 1), *WRKY33* (WRKY transcription factor 33), *MYF6* (myogenic factor 6), *ATF-4* (cyclic AMP-dependent transcription factor ATF-4), *SHOC2* (leucine-rich repeat protein), and *ACS6* (1-aminocyclopropane-1-carboxylate synthase). With regard to pathogenesis-related (PR) proteins, expression of *PR1* genes was upregulated by 22.2-, 5.5-, and 195.4-fold, while one *PR1* gene was downregulated by 2-fold.

With respect to ethylene and JA signal transduction, three genes including *E3.2.1.14* (chitinase), *VSP2* (vegetative storage protein 2), and *GLCAK* (glucuronokinase) were upregulated by 2.1-, 3.0-, and 2.4-fold, respectively. Considering ABA signal transduction, three genes including *PYL* (ABA receptor) and *PRF* (disease resistance protein) were upregulated by 2.1–9.6-fold. In addition, *MAPKKK17_18* (mitogen-activated protein kinase kinase kinase) was downregulated.

The 737 DEGs were introduced into the corresponding biotic stress pathway in Mapman, 308 (41.8 %) of which were enriched in this pathway. Among them, 62 of the 70 (88.6 %) genes related to PR protein, auxin, and cell wall were upregulated, and 39 of the 53 (73.6 %) genes related to signaling and MAPK were also upregulated (Fig. 4). The results of biotic stress pathway analysis were partially consistent with the results of KEGG analysis (Table 1).

Polyphenol biosynthesis There were five enriched KEGG pathways related to the biosynthesis of polyphenols. Twenty-five of the 32 (78.1 %) DEGs involved in phenylpropanoid biosynthesis were upregulated by 2.0–13.1-fold. These genes encode a total of 16 key enzymes, including POD, coniferyl-alcohol glucosyltransferase, and PAL (Fig. S2). Seventeen of the 21 (81.0 %) DEGs involved in flavonoid biosynthesis were upregulated by 2.1–4.4-fold. These genes encode a total of 13 key enzymes, including flavanone 3-dioxygenase, flavonoid 3'-monooxygenase, and naringenin 3-dioxygenase (Fig. S3). All five DEGs involved in flavone and flavonol biosynthesis were upregulated, and they encode flavonoid 3',5'-hydroxylase, flavonol-3-O-L-rhamnoside-7-O-glucosyltransferase, caffeoyl-CoA O-methyltransferase, flavonol 3-O-glucosyltransferase, and flavonoid 3'-monooxygenase, respectively (Fig. S4). Table S5 summarizes the details (gene number, expression change, and protein function) of genes related to polyphenol biosynthesis pathways.

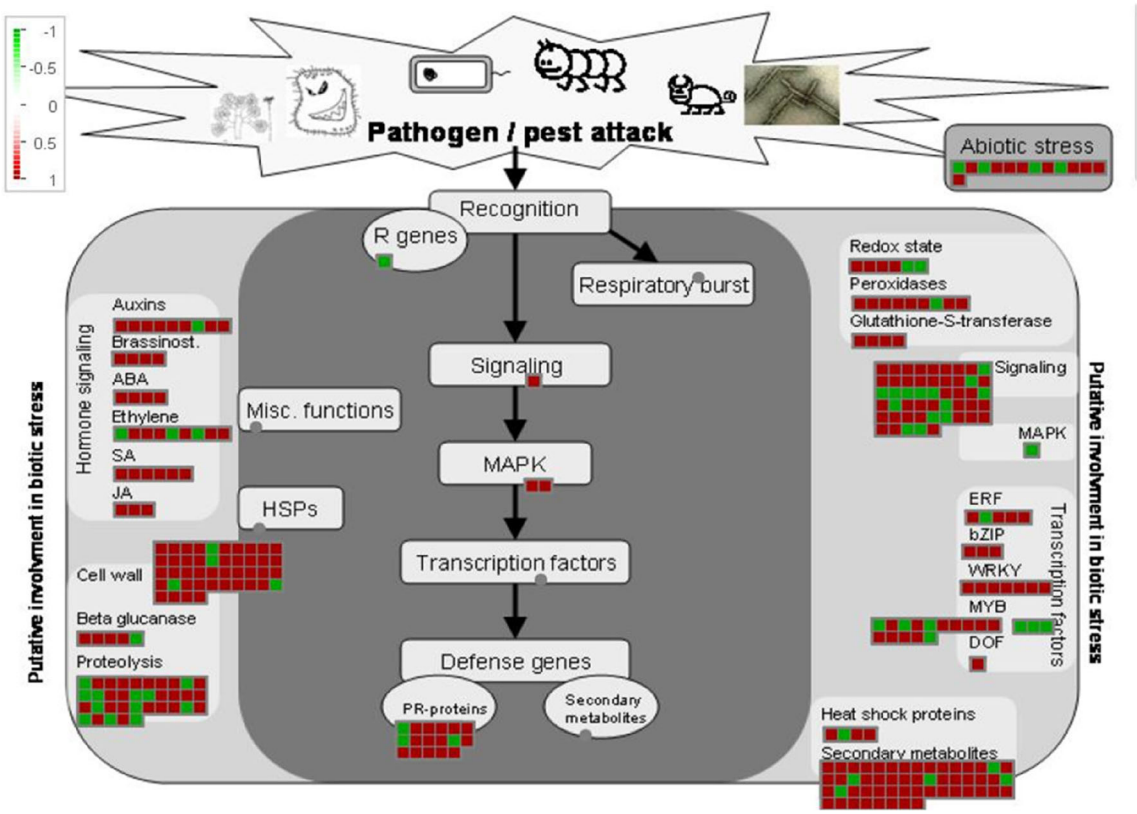

Fig. 4 Biotic stress pathway analysis of differently expressed genes (DEGs) in leaves of tomato plants treated with *P. koreensis* GS. Enrichment of DEGs in the biotic stress pathway was analyzed using MAPMAN, with upregulated (red) and downregulated (green) genes from the treated plants compared with the control plants

Moreover, seven of 10 (70 %) DEGs involved in stilbenoid, diarylheptanoid, and gingerol biosynthesis were upregulated by 2.1–4.4-fold, and they encode four key enzymes, namely shikimate O-hydroxycinnamoyltransferase, caffeoyl-CoA O-methyltransferase, coumaroylquinate (coumaroylshikimate) 3'-monooxygenase, and linoleate 9S-lipoxygenase (Fig. S5). All three DEGs involved in anthocyanin biosynthesis were upregulated, and these genes encode solute carrier family 39 (zinc transporter), member 1/2/3, and flavonol 3-O-glucosyltransferase (Fig. S6).

PCR validation of RNA-Seq results

Eight upregulated DEGs involved in growth regulation, MAPK signaling, and polyphenol biosynthesis were selected for validation by RT-qPCR (Fig. 5). The RT-qPCR data showed that these DEGs exhibited 2.2- to 5.4-fold changes in their expression levels after treatment with strain GS relative to the control group ($P < 0.001$). The expression changes of each gene revealed by RT-qPCR were consistent with those obtained from the RNA-Seq data (Pearson's correlation coefficient $r = 0.707$). For example, the expression changes of *WRKY33*, *E1.11.1.7* (peroxidase), and *PR1* in RT-qPCR analysis were 1.4-, 6.6-, and 4.1-fold ($P < 0.001$), while the corresponding expression changes determined in RNA-Seq analysis were 2.3-, 5.3-, and 5.4-fold, respectively ($P < 0.01$). There were no significant differences in the gene expression changes between RT-qPCR and RNA-seq data ($t = 0.389$, $P = 0.709$). These results indicate that the RNA-Seq data were reliable.

Global metabolomic changes induced by *P. koreensis* GS

Seventy-nine compounds were identified in tomato leaves by GC-MS. There were 25 organic acids, 21 amino acids, one amine, ten sugars, three fatty acids, one nucleotide, four phosphoric acids, eight polyols,

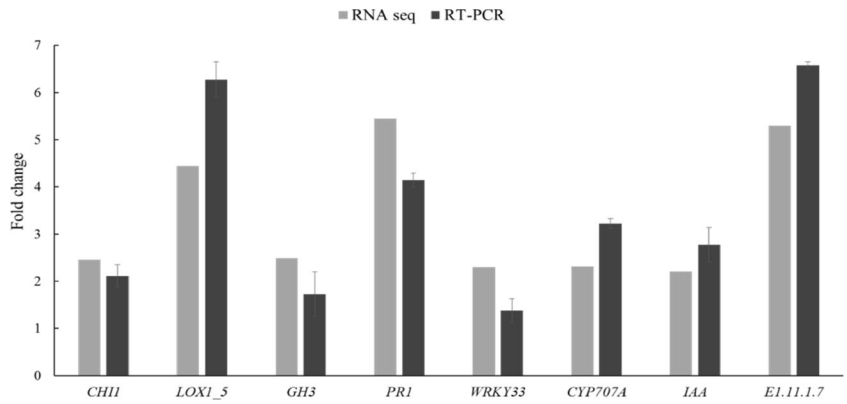

Fig. 5 Expression changes of eight differentially expressed genes involved in plant growth regulation and defense response in tomato leaves for validation of RNA sequencing (RNA-Seq) data by reverse transcription quantitative real-time PCR (RT-qPCR). There were no significant differences in the gene expression changes between RT-qPCR and RNA-seq data ($t = 0.389$, $P = 0.709$; by Student t test)

and six compounds of other categories. Comprehensive analysis showed that the contents of 30 compounds (38.0 %) changed significantly in the treatment group relative to the control group (Fig. 6a), and the details (VIP value, p value, and fold-change) of differential metabolites are listed in Table S6. Among these, 10 organic acids showed significant changes, seven of which were increased. All seven amino acids with significant changes were increased (Fig. 6b).

Links between transcriptome and metabolome

Transcriptome–metabolome co-analysis (Table 2) showed that all the metabolites involved in the phenylpropanoid biosynthesis pathway (which contained 32 DEGs, including 25 upregulated) were enriched after treatment with *P. koreensis* GS. In particular, phenylalanine, tyrosine, and trans-ferulic acid contents were increased by 1.8-, 1.3-, and 1.4-fold in the treatment group compared with the control group, respectively. Moreover, significant changes occurred in one, four, five, and six compounds related to tryptophan metabolism (five DEGs), ABC transporters (three DEGs), 2-oxocarboxylic acid metabolism (seven DEG), and aminoacyl-tRNA biosynthesis pathways (one DEG). The metadata for transcriptome-metabolome co-analysis are summarized in Table S7.

Changes in plant hormones in response to *P. koreensis* GS

Plant hormone analysis showed that mean ABA content in tomato leaves from the control group (113.46 ng g^{-1} fresh weight) was 1.64-fold that in the treatment group (69.34 ng g^{-1} fresh weight). There was no significant difference in SA content between the two groups (355.73 ng g^{-1} vs. 366.69 ng g^{-1} fresh weight). JA was not detected in any group. IAA was detected in the treatment group (9.35 ng g^{-} fresh weight), but not in the control group (below the limit of detection; Fig. 7).

Discussion

PGPR such as *P. koreensis* are soil microbes that colonize plant roots and acquire nutrients from root exudates, which, in turn, benefit plants by increasing tissue growth and/or suppressing disease development (Rafikova et al. 2016). Our recent research showed that the addition of cell-free filtrate of *P. koreensis* GS increased hypocotyl and radicle length of tomato seedlings grown in Petri dishes, while the expression of plant growth-related genes (i.e., expansin genes, *LeEXP2* and *LeEXP18*) in tomato leaves was also upregulated (Guo et al. 2020). In the present study, we verified the effect of *P. koreensis* GS on plant growth promotion using a dilute cell suspension, which effectively increased plant height, stem diameter, and shoot biomass of tomato seedlings grown in pots. Both the culture filtrate and diluted culture of *P. koreensis* GS showed the ability to promote the growth of tomato plants. However, transcriptome analysis revealed that the expression of expansin genes (*LeEXP2* and *LeEXP18*) was not significantly regulated by the diluted culture treatment compared with the control group in the present study. We infer that this discrepancy may be due to the differences of effective bacterial components and plant gene expression patterns over different growth stages of tomato (7 days in the previous study vs. 30 days in the present study). Further, we demonstrated via transcriptome and

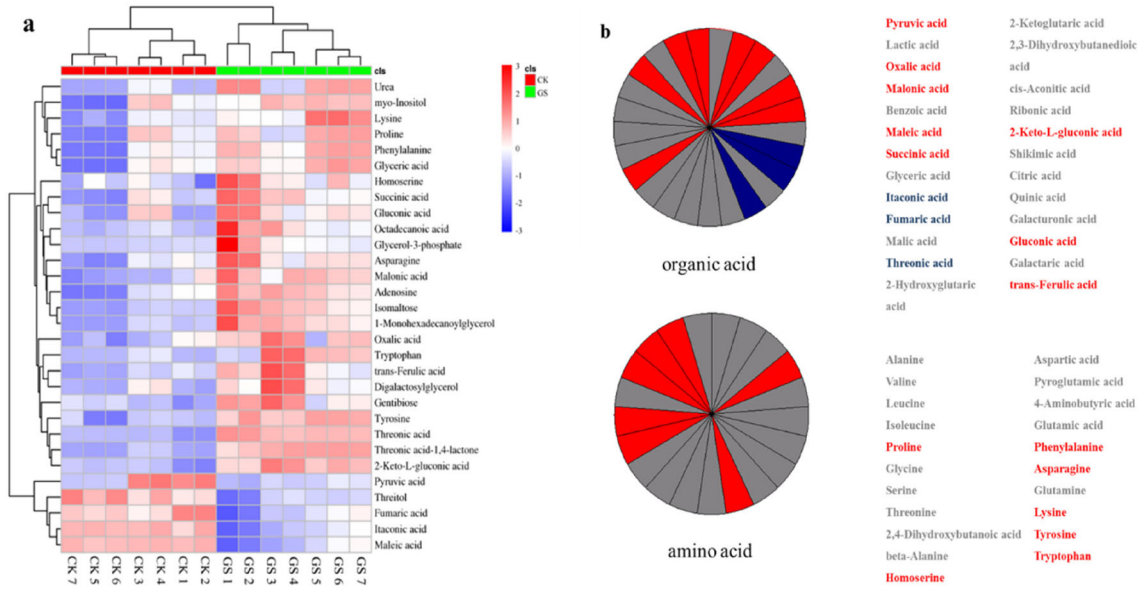

Fig. 6 Metabolomic profiles (**a**) and hierarchical clustering (**b**) of metabolites in the leaves of tomato plants treated with *P. koreensis* GS. Data from seven biological replicates per group are shown. (**a**) Red and blue colors indicate high and low levels, respectively. (**b**) Red color represents significant upregulation, blue color represents significant downregulation, and gray color represents no significant change

metabolome analyses that tomato plants responded to *P. koreensis* GS by modulating multiple growth and defense factors and enhancing biosynthesis of defense metabolites. The results provide insight into the mechanisms underpinning PGPR–plant interactions, and will be useful for field application of potential PGPR such as *P. koreensis* GS in increasing plant stress tolerance.

IAA is a phytohormone that plays a central role in plant growth and development. Our previous research found that *P. koreensis* GS produced IAA in laboratory cultures (Guo et al. 2020). In addition, a preliminary colonization assay indicated that *P. koreensis* GS effectively colonized the root zone, root surface, and root endosphere of tomato plants, with the root surface being an ideal habitat for this bacterium (Note S2; Table S8). In the present study, hormone analysis by LC-MS revealed that following application of *P. koreensis* GS, the IAA content in tomato leaves was much higher than that

Table 2 Transcriptome–metabolome co-analysis in the leaves of tomato plants treated with *P. koreensis* GS

KEGG pathway	Pathway description	DEG count		Metabolites		Metabolite count	
		Up	Down	Up	Down	Up	Down
ko00940	Phenylpropanoid biosynthesis	25	7	Phenylalanine, tyrosine, trans-ferulic acid	-	3	0
ko02010	ABC transporters	2	1	Proline, glycerol-3-phosphate, lysine, urea,	-	4	0
ko00970	Aminoacyl-tRNA biosynthesis	1	0	Asparagine, lysine, proline, phenylalanine, tyrosine, tryptophan		6	0
ko01210	2-Oxocarboxylic acid metabolism	7	0	Lysine, phenylalanine, tyrosine, tryptophan	Pyruvic acid	4	1
ko00360	Phenylalanine metabolism	5	0	Phenylalanine, succinic acid, tyrosine	Fumaric acid, pyruvic acid	3	2
ko00380	Tryptophan metabolism	5	0	Tryptophan		1	0

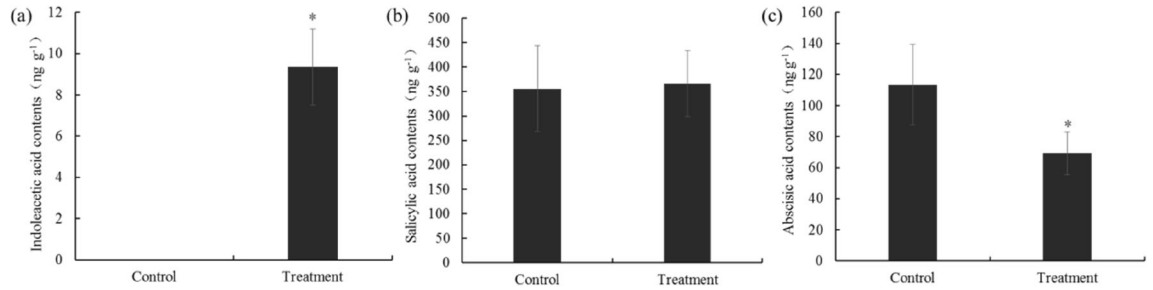

Fig. 7 Hormone contents in the leaves of tomato plants treated with *P. koreensis* GS. (**a**) Indoleacetic acid (IAA); (**b**) abscisic acid (ABA); and (**c**) salicylic acid (SA). Data are the means of seven biological replicates. * $P < 0.05$ compared with the control group (Student's t-test)

in the control group. Taken together, higher concentrations of tryptophan in the leaves and upregulation of auxin early response genes in inoculated plants suggest that the IAA is of plant origin and its biosynthesis is stimulated by the presence of *P. koreensis* GS, or the IAA may be produced by a combination of the bacterium and tomato plants. Further experimentation should be conducted to verify these possibilities.

IAA biosynthesis is mainly divided into tryptophan-dependent and tryptophan-independent pathways (Cohen et al. 2003). However, most important catalytic enzymes and key regulatory genes related to IAA biosynthesis are found in the tryptophan-dependent pathway (Zhao 2010). Here, metabolome analysis by GC-MS indicated that the tryptophan level in tomato leaves was increased by application of *P. koreensis* GS, which might explain the increase of IAA content in plant tissues. Aux/IAA proteins such as IAA1 (Yang et al. 2004) and IAA19 (Kohno et al. 2012) are major components involved in the growth and morphogenesis of plant stems and leaves. In our study, transcriptome analysis by RNA-Seq revealed upregulation of the auxin early response genes *Aux/IAA* and *GH3* in tomato leaves following application of *P. koreensis* GS. In a previous study, *AtIAA19* overexpression in *Arabidopsis* was found to promote the growth of roots, stems, and leaves, and advance flowering (Kohno et al. 2012). Taken together, our results indicate that application of *P. koreensis* GS may promote the growth of tomato plants by facilitating the secretion and signal transduction of auxin.

Ryu et al. (2005) have indicated that GA, brassinosteroid, IAA, and SA signaling pathways are involved in elicitation of plant growth promotion by PGPR such as *P. fluorescens* 89B-61 and *Bacillus subtilis* GB03. In the present study, the transcriptome results revealed upregulation of *GID2* and *PIF3* expression in tomato leaves following application of *P. koreensis* GS. Like GID1 (a GA receptor protein) and DELLA (a negative regulator of GA signaling), GID2 (a protein associated with DELLA protein degradation) is an essential component of GA signaling (Sun 2010; Ueguchi-Tanaka et al. 2007). In addition, PIFs act as negative regulators in light signal transduction, and they not only mediate GA biosynthesis but also participate in GA signal transduction (de Lucas and Prat 2014). GAs can promote the growth and development of plant tissues, especially in key stages such as seed breaking dormancy (Seo et al. 2009), seedling maturation, and vegetative development to reproductive development (Wasilewska et al. 1987). DELLA protein inhibits plant growth in the absence of GA or its presence at low levels, whereas GA binds to the receptor GID1 when cells perceive high levels of GA; this drives GID1 to change conformation and interact with DELLA protein. The GID1–DELLA protein interaction induces DELLA ubiquitination and subsequent degradation, which in turn promotes an increase of free PIFs and their subsequent binding to the target gene promoter. Eventually, GA downstream signaling regulation is activated, thereby facilitating plant growth and development (Hirano et al. 2008; Sun 2010). Moreover, brassinosteroids play a role in vital plant growth and development processes (Li et al. 2002). In this study, application of *P. koreensis* GS induced upregulation of *BAK1* and *TCH4*, genes involved in brassinosteroid biosynthesis. BAK1 is a serine/threonine protein kinase participating in the signal transmission process of brassinosteroids (Li et al. 2002), and TCH4 acts on the major hemicellulose of the plant cell wall (Xu et al. 1995). In summary, our results indicate that the promotion of tomato plant growth by *P. koreensis* GS may be

related to the upregulation of GA and brassinosteroid signaling. In support of our first hypothesis, *P. koreensis* could promote tomato plant growth by facilitating the secretion and signal transduction of specific hormones.

MAPK is a serine/threonine protein kinase involved in plant hormone production, defense gene activation, phytoalexin biosynthesis, and cell wall strengthening (Meng and Zhang 2013). In the present study, five of the eight DEGs involved in FLS2 and BAK1 synthesis were upregulated in tomato leaves after treatment with *P. koreensis* GS. A previous study has shown that BAK1 can phosphorylate FLS2, activating their respective kinase activities (Schulze et al. 2010). Following their activation, FLS2 and BAK1 transmit extracellular signals to plant cells. Then, WRKY33 and PR1 are activated respectively by MKS1 and VIP1 (VirE2-interacting protein 1) to participate in plant disease resistance (Andreasson et al. 2005; Pitzschke et al. 2009). WRKY33 can promote the production of phytoalexin camalexin (Mao et al. 2011), and PR1 is a component of the plant defense system that is closely related to disease resistance. Here, our results showed that 11 of the 12 DEGs associated with downstream components of the MAPK signaling pathway (*MKK4.5*, *VIP1*, *MKS1*, *WRKY22.29*, *WRKY33*, and *PR1*) were upregulated in tomato leaves following application of *P. koreensis* GS. Similarly, Xie et al. (2017) observed that *B. amyloliquefaciens* FZB42 induced expression of several disease resistance genes, including *BAK1*, *FLS2*, *PR1*, and *WRKY29*, in rice leaves. The results indicate that application of *P. koreensis* GS can upregulate the expression of key genes in the MAPK signaling pathway, potentially improving disease resistance in tomato plants.

Polyphenols such as flavonoids, phenylpropanoids, flavones, flavonols, anthocyanins, and lignin are metabolites involved in plant response to biotic and abiotic stresses (Atkinson et al. 2011; Cuong et al. 2018; Moura et al. 2010; Van den Ende and El-Esawe 2014). Polyphenol biosynthesis in vegetative tissues can be induced by specific physiological and environmental factors, thus protecting plants against pathogens, UV radiation (Manuel et al. 2017), low temperature, drought, and high salinity (Catala et al. 2011; Nakabayashi et al. 2014; Shi and Xie 2014). In the present study, the metabolome results showed that application of *P. koreensis* GS induced an increase in the phenylalanine content in tomato leaves. The production of phenolics is mainly through the phenylpropanoid metabolic pathway, which begins with phenylalanine (Cuong et al. 2018). In addition, our analysis of transcriptome results demonstrated that flavonoid biosynthesis, phenylpropanoid biosynthesis, flavone and flavonol biosynthesis, stilbenoid, diarylheptanoid and gingerol biosynthesis, and anthocyanin biosynthesis pathways were all enriched following application of *P. koreensis* GS. In particular, DEGs related to flavone and flavonol biosynthesis and anthocyanin biosynthesis were upregulated in tomato leaves. Trinh et al. (2018) also showed that in the absence of stress conditions, *Paenibacillus pabuli* strain P7S increased the anthocyanin content and biomass of *(A) thaliana* seedlings, along with higher expression of key genes regulating anthocyanin and flavonoid biosynthesis pathways. Moreover, we found that some of the key enzyme-encoding genes in the flavonoid biosynthesis pathway, such as *CHS*, *CHI*, and *F3H*, were upregulated in tomato leaves in the presence of *P. koreensis* GS. The expression of PAL and POD genes involved in phenylpropanoid biosynthesis was increased, which is in agreement with the enhancement of PAL and POD activities we observed in tomato leaves. Martins et al. (2013) reported that without pathogen inoculation, *(B) subtilis* strains UFLA285 and ALB629 increased phenylalanine ammonia lyase activity and phenolic content, while strain ALB629 also facilitated lignin accumulation in bean (*Phaseolus vulgaris*) compared with uninoculated controls. Further, our co-analysis of plant transcriptome and metabolome data showed consistent expression patterns of genes related to the biosynthesis of phenylpropane and associated metabolites. In particular, the metabolites phenylalanine, tyrosine, and trans-ferulic acid were enriched in tomato leaves by application of *P. koreensis* GS. Hence, we found support for our second hypothesis that *P. koreensis* shows potential to induce stress tolerance in tomato plants by enhancing the secretion of polyphenols, and this effect will not affect normal plant growth under non-stress conditions.

Proline can regulate osmotic potential in plant cells and maintain their normal physiological function. Here, the metabolome results showed that the content of eight amino acids, including proline, increased in tomato leaves after treatment with *P. koreensis* GS. Ghosh et al. (2017) also found a PGPR-mediated enhancement of proline turnover in *(A) thaliana* in dehydration conditions. In addition,

wheat plants showed an increase in dry biomass and proline content under different salinity regimes when co-inoculated with two PGPR strains, *(B) subtilis* SU47 and *Arthrobacter* sp. SU18 (Upadhyay et al. 2012). These findings lead us to conclude that *P. koreensis* GS could mitigate drought-induced osmotic stress in tomato plants. Moreover, organic acids secreted by plant roots are often considered to play an essential role in plant tolerance to stresses such as heavy metals, salinity, and drought (Incerti et al. 2007; Fu et al. 2010). In the present study, the contents of six organic acids such as oxalic acid and succinic acid were substantially increased in tomato plants after application of *P. koreensis* GS. The increase of low-molecular-weight organic acids might indirectly enhance plant tolerance to adverse environmental conditions, because they could bind to heavy metals and thereby reduce metal toxicity to the plants (Chen et al. 2015). In addition, plant-derived organic acids play a role in the activation and absorption of soil nutrients. de Werra et al. (2009) found that *P. fluorescens* strain CHA0 secreted gluconate to dissolve mineral phosphate in soil. In summary, the increase of organic acid biosynthesis modulated by PGPR such as *P. koreensis* GS may be beneficial for enhancing plant stress tolerance and improving soil nutrient availability, thereby facilitating plant growth.

Conclusions

This study elaborated complex mechanisms underpinning PGPR–plant interactions based on pot experiments. In tomato, hormone biosynthesis and signal transduction, MAPK signaling, and polyphenol biosynthesis pathways were regulated by the presence of *P. koreensis* strain GS. In addition, metabolites related to plant growth and defense responses (particularly tryptophan, proline, and phenylalanine—the precursor of polyphenols) were enriched by application of the bacterium. This study provides insight into the molecular mechanisms of plant growth promotion by *P. koreensis* and its potential to induce stress tolerance, which may be useful for field application of the microbial agent in tomato production. However, because the present study was conducted under non-stress conditions, the potential for *P. koreensis* to induce plant stress tolerance needs to be verified by further testing under stress conditions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-04837-9.

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Author contributions H.L. and Q.G. conceived the study and proposed the experimental approaches; Y.S., M.S., H.L., Y.L., and Q.G. conducted the experiments; Y.S., Q.G., X.H., and Y.J. contributed to data processing and analysis; and Q.G. and Y.S. wrote the manuscript. All authors read, commented on, and approved the final version of the manuscript.

Compliance with ethical standards All authors are aware with the content of the manuscript and have agreed with the submission to Plant and Soil. The manuscript is not currently being considered for publication in another journal and the manuscript has not been published in whole or in part elsewhere.

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human or animal subjects.

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