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ORIGINAL ARTICLE

Transcriptomic profiling of maize (*Zea mays* L.) seedlings in response to *Pseudomonas putida* stain FBKV2 inoculation under drought stress

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Abstract

Several mechanisms have been proposed for plant growth-promoting rhizobacteria (PGPR)-mediated drought stress tolerance in plants, but little is known about the molecular pathways involved in the drought tolerance promoted by PGPR. We, therefore, aim to study the differential gene response between *Pseudomonas putida* strain FBKV2 and maize interaction under drought stress using Illumina sequencing. RNA Seq libraries were generated from leaf tissue of maize seedlings with and without strain FBKV2 subjected to drought stress. The libraries were mapped with maize genome database for the identification of differentially expressed genes (DEGs). The expression studies confirmed the downregulation of ethylene biosynthesis (ET), abscisic acid (ABA) and auxin signaling, superoxide dismutase, catalase, and peroxidase in FBKV2-inoculated seedlings. On the other hand, genes involved in β-alanine and choline biosynthesis, heat shock proteins, and late embryogenesis abundant (LEA) proteins were upregulated, which could act as key elements in the drought tolerance conferred by *P. putida* strain FBKV2. Another remarkable expression was observed in genes encoding benzoxazinoid (BX) biosynthesis which act as the chemoattractant, which was further confirmed by *gfp*-labeled *P. putida* strain FBKV2 root colonization studies. Overall, these results indicate that secretion of BXs attracted *P. putida* strain FBKV2 resulted in root colonization and mediated drought tolerance by modulating metabolic, signaling, and stress-responsive genes.

Keywords RNA Seq · Transcriptome · Drought tolerance · Maize · *Pseudomonas putida*

Introduction

Plant growth-promoting rhizobacteria (PGPR) are the rhizosphere bacteria that can offer several benefits to host plants such as increasing the availability of nutrients, elimination of deleterious pathogens, and tolerance to abiotic stresses, including drought stress. Several mechanisms have been proposed for PGPR mediated drought stress tolerance in plants. Phytohormone producing *Azospirillum* sp. enhanced plant growth promotion and induced drought tolerance in wheat (Arzanesh et al. 2011). 2R, 3R-butanediol producing *Pseudomonas chlororaphis* O6 induced stomatal closure genes and reduced water loss in *Arabidopsis thaliana* (Cho et al. 2008). Similarly, 1-aminocyclopane-1-carboxylate (ACC)-deaminase producing *Achromobacter piechaudii* ARV8 lowered ethylene levels and induced drought tolerance in tomato and pepper seedlings exposed to drought stress (Mayak et al. 2004). PGPR *P. putida* strain GAP-P45 enhanced plant biomass, relative water content, and leaf water potential by the accumulation of proline in maize seedlings exposed to drought stress (Sandhya et al. 2010). Exopolysaccharides producing *Rhizobium leguminosarum* (LR-0), *Mesorhizobium ciceri* (CR-30 and CR-39), and *Rhizobium phaseoli* (MR-2) showed beneficial interaction to wheat (non-legume) under drought stress (Hussain et al. 2014). Furthermore, *Bacillus* sp. enhanced drought tolerance in maize seedlings by reducing the activity of the antioxidant enzymes such as ascorbate peroxidase (APX) and glutathione peroxidase (GPX) (Vardharajula et al. 2011).

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Gene expression profiling has also been used to characterize a variety of genes expressed under drought stress in plants inoculated with PGPR (Kandasamy et al. 2009; Yuwono et al. 2005). Increased mRNA transcripts of a drought-response gene, EARLY RESPONSE TO DEHYDRATION 15 (ERD15) in *Paenibacillus polymyxa* B2-inoculated *A. thaliana* enhanced drought tolerance (Timmusk and Wagner 1999). Six differentially expressed stress proteins were identified in pepper plants inoculated with *Bacillus licheniformis* K11 under drought stress (Lim and Kim 2013). Stress-related genes APX1, SAMS1, and HSP17.8 were induced in wheat inoculated with *Bacillus amyloliquefaciens* 5113 and *Azospirillum brasilense* NO40 (Kasim et al. 2013) respectively. Jasmonic acid marker genes, salicylic acid-regulated genes, and the ethylene-response genes were upregulated in *A. thaliana* colonized with *P. chlororaphis* O6 (Cho et al. 2013). Recent studies with Illumina sequencing (HiSeq 2000 system) revealed abscisic acid (ABA) signaling genes in sugarcane cv. SP70-1143 inoculated with *Gluconacetobacter diazotrophicus* conferred drought tolerance (Vargas et al. 2014).

Our previous work has shown that the potential of *P. putida* strain FBKV2 and GAPP45 colonization-enhanced drought tolerance in maize by the accumulation of cellular metabolites reduced stomatal conductance and antioxidant enzymes (Vurukonda et al. 2016; Vardharajula et al. 2011; Sandhya et al. 2010). Till date, little is known about the molecular pathways involved in the drought tolerance promoted by PGPR that colonize the maize. We, therefore, hypothesized that rhizobacteria inoculation alter the expression of metabolic, signaling, and stress-protective genes in maize leaves. To address this hypothesis and to advance our understanding of maize response to drought stress with and without bacterial inoculation, we adopted RNA Seq transcriptome analysis using Illumina deep sequencing of maize seedlings.

Material and methods

Bacterial strain

In our previous studies, *Pseudomonas* spp. strain FBKV2, isolated from eggplant (*Solanum melongeana* L.) rhizosphere, showed multiple PGP traits under both non-stress and drought stress conditions and enhanced drought tolerance in maize. The strain was identified as *Pseudomonas putida* by 16SrRNA sequence analysis, and the sequence was submitted to GenBank under the accession number KT311002.1 (Vurukonda et al. 2016).

gfp labeling of *P. putida* strain FBKV2

The derivative of broad-host-range vector pBBR1MCS-5 (Stuurman et al. 2000), which contains the green fluorescent protein (*gfp*) gene under the control of *lac* promoter with gentamycin (Gm) resistance, was obtained from Plant Microbe Interaction lab, Centre for Cellular and Molecular Biology (CCMB), Telangana State, India. The wild-type *P. putida* strain FBKV2 was grown in 5 mL of Luria Bertani (LB) medium at 28 °C under continuous shaking (120 rpm) until the optical density of 0.6 at λ 600 nm. The cells were harvested by centrifugation at 5000 rpm from 5 min, resuspended in 5 mL of cold sterile water, and centrifuged at 5000 rpm for 5 min. The supernatant was discarded, and the pellet was washed twice (5000 rpm for 5 min) with 10% glycerol. Transformation was performed by electroporation (Gene Pulser, Bio-Rad) in an electroporation cuvette (0.2 cm) containing 100 μL of competent cells (*P. putida* strain FBKV2), plus 2 μL plasmid DNA (100 ng μL^{-1}). The following pulse conditions were applied: 12.5 kV cm^{-1}, 25 μF, and 200 Ω (Krzyzanowska et al. 2012a, b; Choi et al. 2006). After transformation, 1 mL of LB medium was added; the mixture was incubated for 1 h at 28 °C and plated on to LB agar medium supplemented with gentamycin (40 μg mL^{-1}) and incubated for 48 h at 28 °C. The identification and selection of clones carrying the *gfp* gene were carried out under UV light.

Plant material and growth conditions

A pot experiment was conducted under greenhouse condition to compare the efficacy of selected bacterial strain *P. putida* strain FBKV2 (*gfp* labeled) for promoting the growth of maize under drought stress conditions. The soil used for pot experiments belongs to the Chalkas series and has been classified as "red earths with loamy subsoil" in the Indian soil classification system, which falls under the order Alfisols (Bhattacharyya et al. 2007). The soil was collected from the homogeneous horizon (0–20 cm) of College Farm (Field soil), PJTSAU Campus, Rajendranagar, Hyderabad, India, a semi-arid region under rain-fed production system. The soil was air-dried and sieved (<2 mm) before being analyzed for the physicochemical properties. The soil contained 73.2% sand, 5.6% silt, 21.2% clay with 1.43 Mg m^{-3} bulk density, 2.52 Mg m^{-3} particle density, 38.4% total porosity, and 39.2% water holding capacity; it had a pH of 6.8 with an electrical conductivity of 0.13 dms; the soil contain Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} in the ratio of 3.2, 2.1, 0.31, and 0.25 cmol (p+)/kg with CEC of 16.2 cmol (p+)/kg. The organic C, total N, and total P content of soil were 0.89, 0.13, and 0.09 g/kg, respectively (Vurukonda et al. 2016).

Maize (*Zea mays* L. var. DHM 117) seeds were surface sterilized with 0.1% $HgCl_2$ (30 s) followed by 70% ethanol for 1 min and six times of repeated washing with sterile water (1 min each time) (Vurukonda et al. 2016). The effectiveness of surface sterilization was checked by plating the seeds and aliquots of the final rinse onto nutrient agar (NA) plates. Seeds

were considered positive for sowing when no colonies were observed on the NA plates after incubation for 48 h at 28 °C. Surface-sterilized seeds were treated with *gfp*-labeled *P. putida* strain FBKV2 suspension (10^9 cfu mL^{-1}) for 30 min, and for control, seeds were incubated in sterile distilled water.

Four seeds were sown in pots (surface sterilized) with diameter 17 cm and height 15 cm containing 1.5 kg of sterile soil. Pots were arranged in the greenhouse using a completely randomized design with three replications of each treatment. After 1 week of germination, the seedlings were thinned to two per pot. Soil water content was 15.9% (− 0.3 MPa), determined according to Sandhya et al. (2009) by drying the initially saturated soil at different matric potentials by pressure plate apparatus (Model-1250, Santra Barbara, CA, USA). The soil water content of the pots was maintained at 100% water holding capacity (WHC) during the experiment by daily sprinkling with sterile distilled water, and the nutrients (Hoagland's no. 2 basal salt mixture, Sigma-Aldrich) were supplied on a weekly basis. Drought stress was induced by discontinuing water after 14 days of planting. After stopping irrigation, plants were observed for signs of wilting and leaves were harvested for further studies (4 days of drought stress).

Leaf relative water content

For measuring relative water content (RWC), the leaves were cut into small discs of 1.5 cm^2 and fresh weight (FW) was recorded immediately, followed by hydrating the sample overnight by immersing in water and turgid weight (TW) was recorded after blotting the leaf sample gently. The samples were dried at 70 °C until constant dry weight (DW) was observed. RWC was calculated according to the formula RWC (%) = [(FW − DW) / (TW − DW)] × 100 (Teulat et al. 2003).

Specimen preparation for microscopy

To validate the establishment and colonization of strain FBKV2 on the roots of maize seedlings, root colonization studies were performed using confocal laser scanning microscopy (CLSM). Maize root specimens were prepared by scratching a piece of the root surface, around 1 cm in length, from different parts of the root with a sterile blade. All specimens were softly rinsed with sterile distilled water prior to merging into phosphate-buffered saline (PBS) for microscopic observation.

Confocal laser scanning microscopy

CLSM was performed at Banaras Hindu University, Varanasi, India, with a Carl Zeiss Microscopy LSM780, Axio Imager 2 system. While GFP fluorescence was recorded by using an excitation laser of 488 nm (argon laser) and collecting the emission of 500–600 nm, an excitation with NeHe laser of 561 nm was used and the emission band of 538–624 nm was collected. Images were acquired and reconstructed by Zen 2012 Software.

RNA Seq

RNA isolation and library preparation

Drought stress was induced by discontinuing water after 14 days of planting. After stopping irrigation, plants were observed for signs of wilting. When shrinkage of leaves and stem was clearly visible, plants were harvested (4 days of drought stress) and the youngest leaf (fourth leaf) was collected in RNA*later* (RNA stabilizing reagent, Qiagen) and used for Illumina deep sequencing. Two biological replicates were used for all RNA Seq experiments. The total RNA from the leaf tissues was extracted using Trizol reagent (Invitrogen) and purified using the RNeasy Plant Mini kit (Qiagen). The integrity and quality of the total RNA were checked by a NanoDrop, formaldehyde-agarose gel electrophoresis, and Agilent 2100. The poly(A) RNA was isolated from purified total RNA using Oligo (dT) beads. Following purification, the mRNA was fragmented randomly into small pieces using fragmentation buffer, and the cleaved RNA fragments were copied into the first-strand cDNA using reverse transcriptase and random hexamer primers. Second-strand cDNA synthesis was done using synthesis buffer (Illumina), dNTPs, DNA polymerase I and RNaseH, and the cDNA fragments were processed for end repair, an addition of a single "A" base, and ligation of the adapters. These products were then purified and enriched by PCR to create the final cDNA library (NEBNext® Ultra™ RNA Library Prep Kit for Illumina®) and sequenced on the HiSeq 2500 sequencer according, to the manufacturer's recommendations (Illumina). Paired end sequencing with 2 × 150 bp read length was performed with multiplexed sampling assay. All the RNA Seq experiments were performed at Nucleome Informatics Private Limited, Hyderabad, Telangana State, India.

Raw data filtering

DrSeq™ Software suite was used to execute the RNA Seq data analysis. The filtering process was done by Trimmomatic and includes (1) removal of reads containing adapters, (2) removal of reads containing $N > 10\%$ (N represents the base cannot be determined), and (3) removal of reads containing low quality (Q score ≤ 5) base which is over 50% of the total base. The detailed statistics for the quality of sequencing data of four libraries (presence of *P. putida* strain FBKV2 (DS + PP) and uninoculated control (DS) in two replicates each) is shown in Supplementary Table S1.

Mapping reads to a reference genome and transcript assembly

The filtered reads were mapped to the maize genome (http://ftp.ensemblgenomes.org/pub/plants/release-28/fasta/zea_mays/dna/Zea_mays.AGPv3.28.dna.toplevel.fa.gz) using Tophat version v2.0.12 (Kim et al. 2013), which allows the identification of novel exons and novel intergenic transcripts. The mismatch parameter is set to two, and other parameters are set to default. The overview of mapping status of four libraries is shown in Supplementary Table S2.

The Map files generated by Tophat version v2.0.12 were provided as an input file to the software Cufflinks version v2.1.1, which assembles the alignments in the Map file into transfrags. The Cufflinks parameters are set to default. The assembled transfrags are then compared to the reference transcripts to determine if they are sufficiently different to be considered novel.

Gene expression quantification

The gene expression levels were estimated by counting the reads that map to genes or exons. In order for the gene expression levels estimated from different genes and experiments to be comparable, the FPKM (Fragments Per Kilobase of transcript sequence per Million) was used. FPKM is the commonest method of estimating gene expression levels, which considers the effects of both sequencing depth and gene length on-counting of fragments (Trapnell et al. 2010). HTSeq version v0.6.1 (Anders et al. 2015) software was used to analyze the gene expression levels, using the union mode. An FPKM value of 1 was set as the threshold for determining whether the gene is expressed or not. The gene expression data generated by HTSeq was provided as an input file to study the differential gene expression (DEG) using DESeq R package (1.18.0) (Anders and Huber 2010) with padj < 0.05.

GO enrichment

Gene ontology (GO) classification was carried out using BLASTx results in GO Seq, Release 2.12 software for functional classification of GO terms (Young et al. 2010). GO terms were obtained from nr hits using GO Seq software with default parameters during mapping.

KEGG pathway enrichment analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis identifies significantly enriched metabolic pathways or signal transduction pathways associated with differentially expressed genes compared with the whole genome background. KOBAS v2.0 with corrected P value < 0.05 was used to study the KEGG pathway enrichment analysis.

MapMan analysis

The MapMan software (Usadel et al. 2009) was used to identify the metabolic pathways of interest annotated by groups of participatory entities (maize transcripts), where each entity within a given group is represented by a discrete signal visualized using intensity of the color (Thimm et al. 2004).

qRT-PCR analysis

Real-time PCR was carried out using a Bio-Rad CFX96 Touch Real-Time thermocycler. The comparative CT method of quantitation was performed using maize *Actin 2* and actin-binding protein (*Zmabp3*) as reference genes. Total RNA was extracted from two biological replicates of each sample and converted to cDNA using oligo-dT and random primers mix using QuantiTect Reverse Transcriptase (Qiagen). Three technical replicates of each biological replicates were used for qRT-PCR using QuantiFast SYBR Green PCR Kit (Qiagen). Experiments were performed on five to nine log dilutions of each of the target genes together with the reference genes for equal amplification efficiencies. To each well, 2.5 μL of first strand cDNA, 12.5 μL of SYBR Green solution, 2.5 μL of the forward primer (10 μM), and 2.5 μL of reverse primer (10 μM) were added, along with 5.0 μL of sterile, ultrapure water to bring the final volume to 25 μL in each well. Negative control reactions using untranscribed RNA were also run to confirm the absence of genomic DNA. To determine relative fold differences for each sample in each experiment, the CT value for each gene was normalized to the CT value for the reference gene and was calculated relative to a calibrator using the ΔΔCT method as described (Livak and Schmittgen 2001). Primers used for qRT-PCR were designed with Integrated DNA technologies (https://eu.idtdna.com/site/order/qpcr/predesignedassay). The gene sequence used for primer design of each transcript was aligned to the NCBI maize database, and the transcripts with specific regions were selected for designing primers (Table S3).

Data availability The sequence data (raw data) generated in this study have been deposited at NCBI Sequence Read Archive (SRA) database (www.ncbi.nlm.nih.gov/sra). The Bioproject accession number for maize seedlings transcriptome is PRJNA362689, and SRA experimental accession numbers are SRX2510677 and SRX2510673.

Results

Evaluation of *P. putida* stain FBKV2 for growth promotion of maize seedlings under drought stress

In order to investigate the beneficial effects of *P. putida* strain FBKV2 to maize plants, the length and fresh weight of roots and shoots were evaluated 1 day before drought stress. Leaf samples were labeled as drought stress inoculated with *P. putida* strain FBKV2 (DS + PP) and uninoculated control under drought stress (DS). Bacterial inoculation significantly enhanced the seedling growth compared to non-inoculated plants (root, shoot, and biomass data not shown). As physiological modifications are the first responses of plants to overcome water deficit, phenotypic analysis of maize seedlings was monitored under drought stress. As shown in Fig. 1a, seedlings started showing visible signals of stress, such as rolling and wilting of leaves, after 4 days of drought stress. The visible signals of stress were lower in inoculated seedlings compared to uninoculated treatment. Furthermore, survival rate rapidly decreased in uninoculated treatment and seedlings died completely at the end of sixth day. However, seedlings inoculated with *P. putida* strain FBKV2 survived up to 10 days after exposure to drought stress and started wilting thereafter. These results suggest that inoculation with *P. putida* strain FBKV2 enhanced drought tolerance in maize seedlings compared to non-inoculated seedlings. The positive influence of microbial inoculations was also observed on leaf relative water content (RWC) content. Inoculation with *P. putida* strain FBKV-2 significantly increased the RWC (71.25 ± 1.05) over uninoculated control (66.05 ± 1.28).

Root colonization of maize seedlings by strain FBKV2

To visualize the root colonization of maize seedlings by *gfp*-labeled *P. putida* strain FBKV-2 under drought stress, root specimens were viewed by CLSM. As shown in Fig. 1b, the fluorescent rod-shaped structures of strain FBKV2 indicated the presence of high numbers of bacterial cells compared to uninoculated seedlings (Fig. 1c), validating the successful root colonization by *P. putida* strain FBKV2.

RNA Seq analysis of maize seedlings

Between 39 and 52 million reads from each sample were generated using Illumina HiSeq 2500 technology. The high-quality clean reads (uniquely mapped reads; Table S1) were used for mapping to the reference genome and the gene expression level was estimated by FPKM. Figure 2a, showing the violin plot of the log FPKM values, suggests that the quartile and the median values among the samples being compared for differential gene expression are almost identical with a slightly higher density in *P. putida* strain FBKV2-inoculated treatment. Similarly, the magnitude of variability among the biological replicates was also determined using Pearson correlation coefficient method. The closer the correlation coefficient is to 1, the greater the similarity of the samples. In the present RNA Seq analysis, the R^2 is 0.918 (Fig. 2b), indicating slight variability among the biological replicates.

In the present study, cluster analysis was also performed to identify the genes with similar expression patterns under various experimental conditions. From the cluster analysis (Fig. 2c), it is evident that the maize seedlings inoculated with *P. putida* strain FBKV2 showed differential expression pattern than the uninoculated seedlings under drought stress. A total of 3738 DEGs were obtained. Among them, 1163 genes showed increased transcript abundance and 2575 genes showed decreased transcript abundance in *P. putida* strain FBKV2-inoculated seedlings (DS + PP) (Fig. 3a).

A comparable gene expression was seen between inoculated and uninoculated treatments in gene ontology (GO) terms related to plant growth and development. GO categories

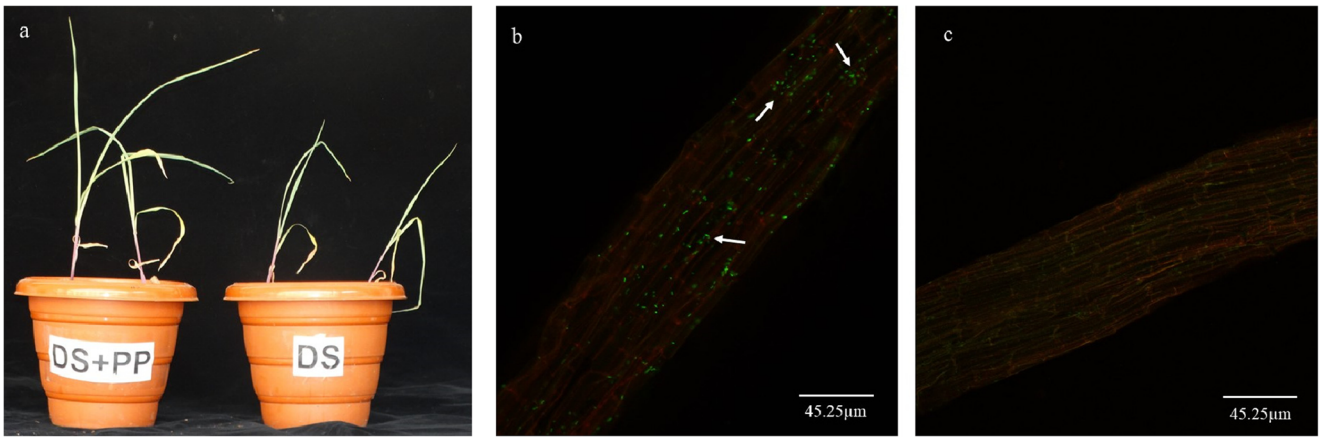

Fig. 1 **a** Phenotype of maize seedlings under drought stress (after 4 days withholding water) inoculated with *P. putida* strain FBKV2 (DS + PP) or uninoculated (DS). **b** CLSM micrographs of *gfp*-labeled *P. putida* strain FBKV2 colonizing maize root. **c** Uninoculated control root. Arrows indicate fluorescent bacteria colonizing maize root surface. Bar represent the scale of measurement

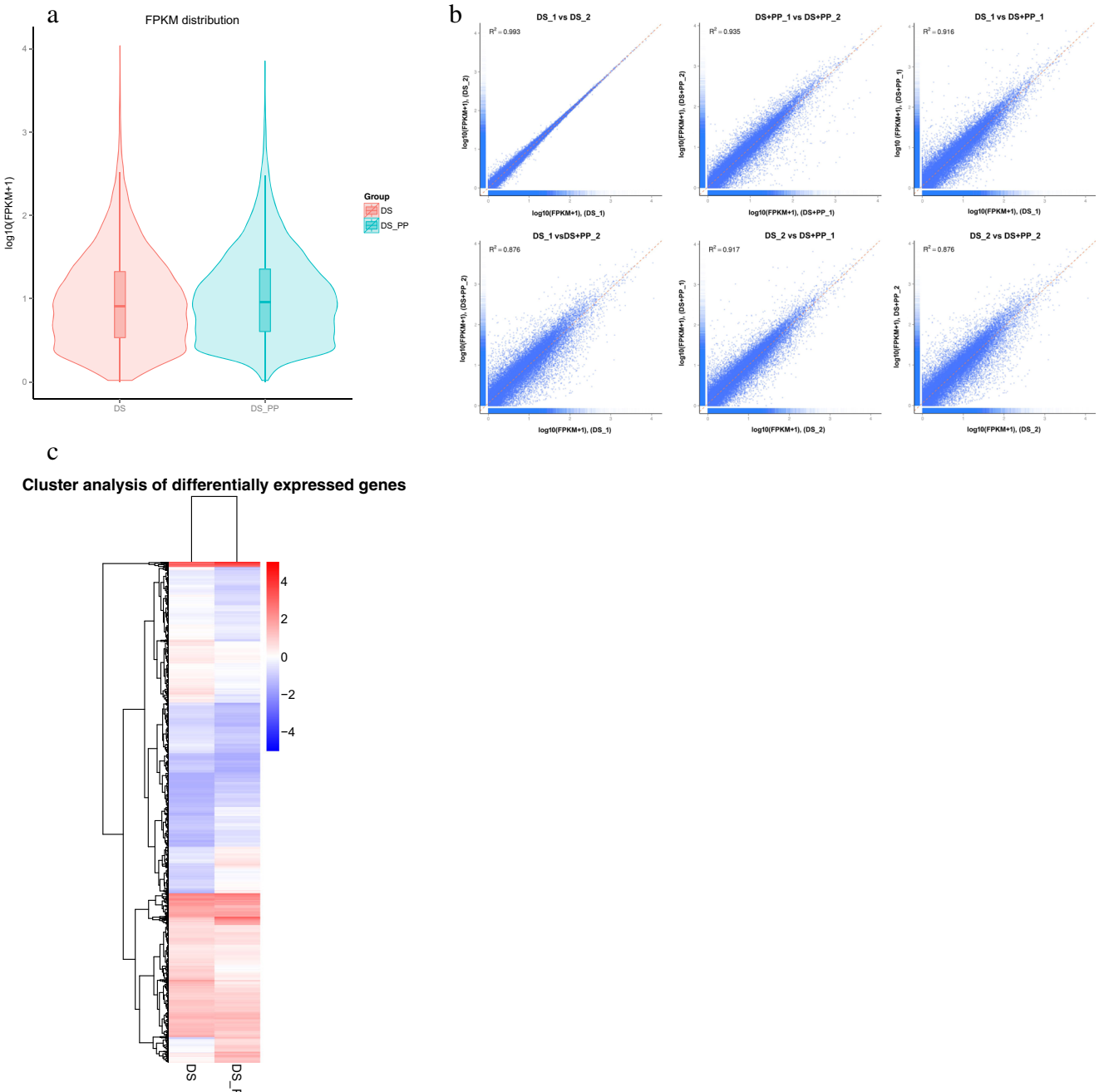

Fig. 2 **a** Violin plot of the log Fragments Per Kilobase of transcript sequence per Million base pair-sequenced (FPKM) expression value. The *x*-axis shows the sample/library names, and the *y*-axis shows the log10(FPKM + 1). Each violin has five statistical magnitudes (max value, upper quartile, median, lower quartile and min value). In the present experiment, the quartile values are almost identical among the replicates. The violin width shows the gene density. The gene density is higher in DS + PP sample. **b** The overall results of FPKM cluster analysis, clustered using the log10(FPKM + 1) value. The scatter diagrams demonstrate the correlation coefficient between samples; R^2, the square of the Pearson coefficient. In the present experiment, the R^2 is 0.918, indicating slight variability among the biological replicates. **c** Heat maps of the correlation coefficient between samples. Red denotes genes with high expression levels, and blue denotes genes with low expression levels. The color ranges from red to blue represents the log10 (FPKM + 1) value from large to small

corresponding to various aspects of the biological process such as response to abiotic stress, water stress, water deprivation, abscisic acid, response to hormones, and biological activity were enriched in *P. putida* strain FBKV2-inoculated seedlings (DS + PP) compared to uninoculated (DS) control (Fig. 3b). Similarly, GO categories related to molecular function (catalytic, glutathione transferase, oxidoreductase activity) and cellular components (cell wall, cell periphery, non-membrane bound organelle, and ribonucleoproteins) were significantly enriched with increased transcript abundance in DS + PP treatment compared to DS (Fig. 3b). Furthermore, MapMan software tool was employed to analyze the

statistically significant drought-mediated gene expression data for *P. putida* strain FBKV2-inoculated seedlings (Fig. 4) for known metabolic pathways, biological processes, and functional categories. Few of the GO categories were selected for detailed examination.

Differential expression of carbohydrate metabolism-associated genes

In the present study, carbohydrate metabolic genes showed altered expression pattern in maize seedlings inoculated with *P. putida* strain FBKV2 under drought stress. Genes involved in starch synthesis namely plastid ADP-glucose pyrophosphorylase large subunit (GRMZM2G391936), the first step in starch biosynthesis, showed decrease gene expression. Similarly, granule-bound starch synthase-1 (GRMZM2G024993) responsible for amylose synthesis also showed decreased transcript abundance, whereas starch branching enzyme-III (GRMZM2G005298) responsible for amylopectin synthesis showed increased transcript abundance. On the other hand, a large number of genes involved in the starch break down such as β-amylase isoforms (GRMZM2G450125, GRMZM2G035749, GRMZM2G082034, GRMZM2G007939, and GRMZM2G025833) showed decreased transcript abundance, whereas α-amylase (GRMZM2G138468) showed increased gene expression (Table 1).

In sucrose metabolism, genes encoding sucrose synthase 7 (GRMZM2G410704) and sucrose phosphate phosphatase (GRMZM2G097641) showed increased transcript abundance (Table 1). These data suggest that starch biosynthesis and degradation have been suppressed and sucrose accumulation has been enhanced, and at the same time, its degradation into UDP-glucose and fructose serves as a starting point for glycolysis and synthesis of cellulose.

Differential expression of membrane transporters

The dynamic GO enrichment results revealed that a large number of genes belonging to the "membrane transporters" family significantly downregulated in response to *P. putida* strain FBKV2 inoculation under drought stress (Table 2). The most important functional group of this category is the aquaporin genes. Out of nine genes, six genes annotated as

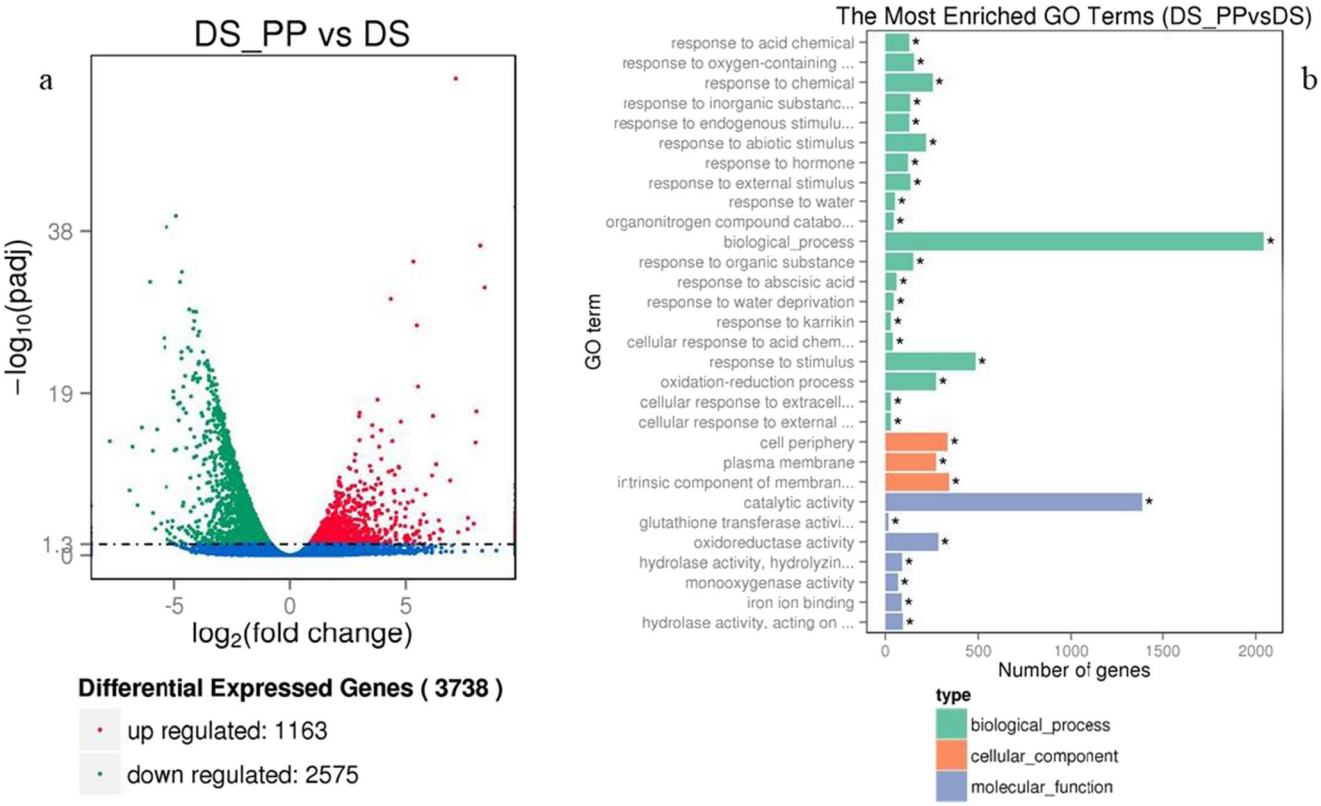

Fig. 3 **a** Volcano plot for differentially expressed genes. The *x*-axis shows the fold change in gene expression between different samples, and the *y*-axis shows the statistical significance of the differences. Significantly up- and downregulated genes are highlighted in red and green colors, respectively. Genes did not express differently between treatment group and control group are in blue. **b** Gene ontology functional classification. The *x*-axis is the GO terms enriched, and the *y*-axis is the number of differential expression genes. Different colors are used to distinct biological process, cellular component, and molecular function, in which the enriched GO terms are marked by asterisk

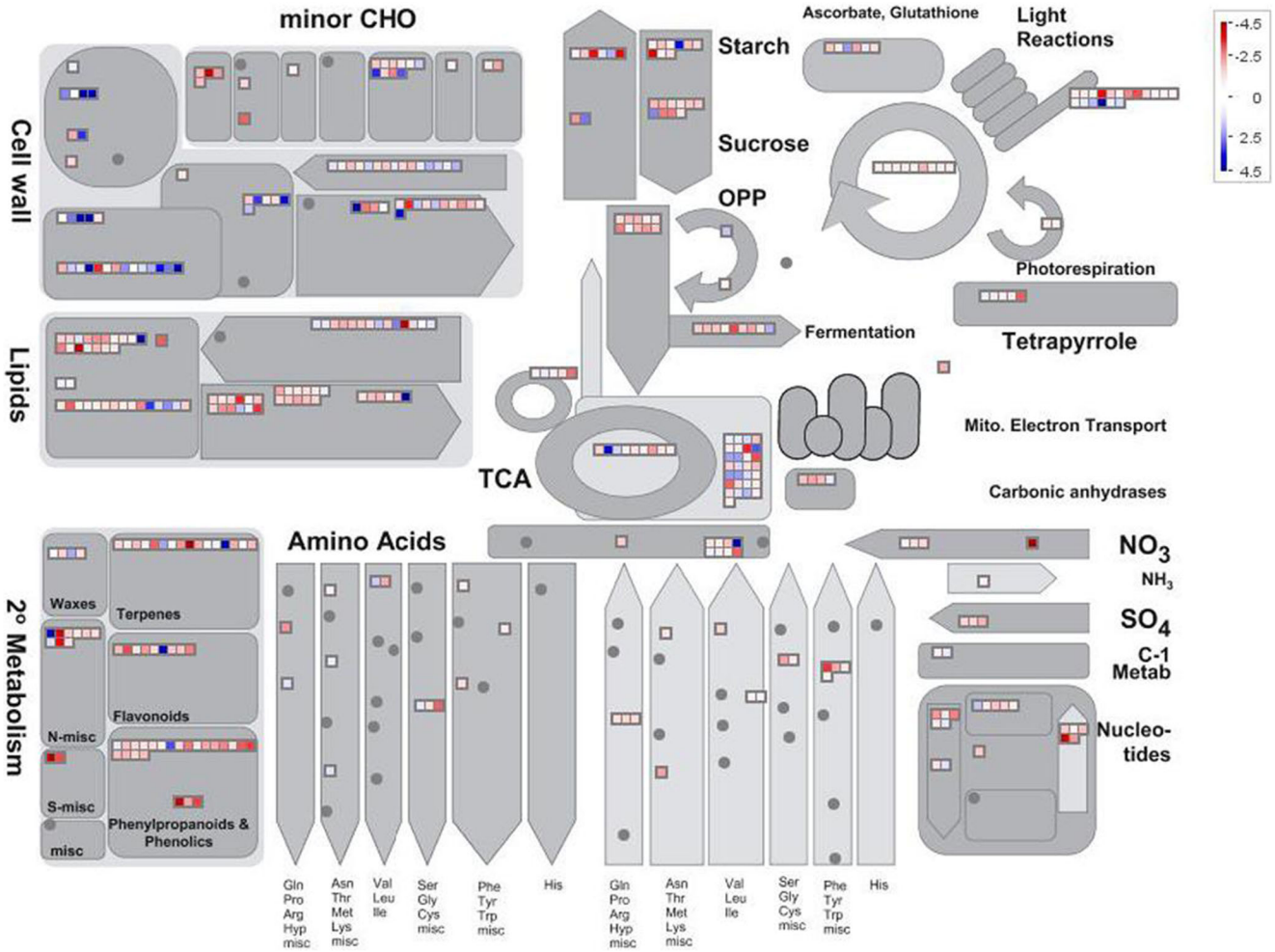

Fig. 4 MapMan overview of differentially expressed transcripts involved in different metabolic processes under drought stress in *P. putida* strain FBKV2 (DS + PP) treatment. The images were obtained using MapMan software, showing different functional categories that passed the cutoff (less than 0.05 *Q* value and greater than 2-fold change) for differential expression. Red stands for downregulation, and blue stands for upregulation

plasma membrane intrinsic proteins (PIPs), one as tonoplast intrinsic proteins (TIPs) and two genes correspond to NOD26-like intrinsic proteins (NIPs). Among the nine aquaporins, gene-encoding PIP2–4 (GRMZM2G154628) of PIP family showed increased transcript abundance, whereas the remaining eight aquaporins showed decreased transcript abundance (Table 2). This result suggested that strain FBKV2 inoculation downregulated aquaporins to minimize the water flow through cell membranes and increase the leaf turgor, which might have enhanced the cell membrane stability with higher relative water content (71.25 ± 1.05) in the seedlings compared to uninoculated control (66.05 ± 1.28).

Differential expression of abiotic stress-responsive genes

The maize expression data annotated by GO category "response to abiotic stimulus" were significantly affected by *P. putida* strain FBKV2 inoculation. Among the reactive oxygen species (ROS) scavenger genes, three genes encoding glutathione *S*-transferase (GRMZM2G077206, GRMZM2G096269, GRMZM2G363540), and gene-encoding dehydroascorbate reductase—DHAR4 (GRMZM2G005710)—showed increased transcript abundance; three superoxide dismutase CU-ZN family isomers (GRMZM2G169890, GRMZM2G025992, GRMZM2G058522), catalase isoenzyme B (GRMZM2G088212), and putative glutathione peroxidase (GRMZM2G144153) showed decreased transcript abundance (Table 3). Another important functional group of GO category "response to abiotic stimulus" is heat shock proteins. In the present study, genes encoding chaperonin cpn60 (GRMZM2G042253, GRMZM2G074790), HSP40 (GRMZM2G049373), chaperonin cpn10 (GRMZM2G091189), HSP70 (GRMZM2G137696), and 18.8 kDa class V HSP (GRMZM2G429396) showed

Table 1 Effects of drought stress on the expression of genes associated with starch and sucrose metabolism in maize seedlings inoculated with *P. putida* strain FBKV2 (DS + PP)

Maize ID	Annotation	log2-fold change
Starch metabolism		
GRMZM2G391936	Plastid ADP-glucose pyrophosphorylase large subunit	− 0.8473
GRMZM2G024993	Granule-bound starch synthase 1	− 3.5877
GRMZM2G005298	Starch branching enzyme III	+ 1.2573
GRMZM2G450125	Beta-amylase 1	− 1.8337
GRMZM2G035749	Beta-amylase	− 0.9810
GRMZM2G138468	Alpha-amylase	+ 3.6869
GRMZM2G082034	Beta-amylase	− 1.8607
GRMZM2G007939	Beta-amylase 3, chloroplastic-like	− 1.5131
GRMZM2G025833	Beta-amylase	− 3.9642
Sucrose metabolism		
GRMZM2G410704	Sucrose synthase 7	+ 1.9681
GRMZM2G097641	Sucrose phosphate phosphatase (SPP2)	+ 2.6313

− stands for downregulation, and + stands for upregulation

increased expression (Table 3). Furthermore, genes involved in β-alanine biosynthesis such as polyamine oxidase (GRMZM2G150248), along with genes associated with choline biosynthesis such as putative phospho ethanolamine *N*-methyltransferase (*PEAMT*) (GRMZM2G122296), showed increased transcript abundance (Table 3) in *P. putida* strain FBKV2-inoculated seedlings compared to uninoculated.

Overall, these data suggest that genes encoding chaperonins, β-alanine, choline biosynthesis, and few ROS genes were more induced by strain FBKV2 inoculation and might have played important roles in seedling tolerance to drought stress.

Differential expression of hormonal metabolism-associated genes

In the present study, differential expression pattern of genes for biosynthesis, signaling, and response to hormones was analyzed. A large number of genes involved in the biosynthesis and signaling of ABA in maize seedlings colonized by *P. putida* strain FBKV2 were mainly repressed. This repression was indicated by the downregulation of pyrabactin resistance-like protein 3 (GRMZM2G154987), bzip transcription factor ABI5 (GRMZM2G479760), and protein phosphatase family proteins (GRMZM2G134628, GRMZM2G059453, GRMZM5G818101, GRMZM2G001243, GRMZM2G300125, GRMZM2G159811, GRMZM2G082487, and GRMZM2G177386), whereas three genes encoding serine/threonine protein kinase (GRMZM2G147051, GRMZM2G147857 and GRMZM2G084806) showed upregulation (Table 4).

A remarkable effect was observed in the genes associated with ethylene (ET) biosynthesis and signaling. Two genes encoding 1-aminocyclopropane-1-carboxylate oxidase 1 (GRMZM2G052422) and 1-aminocyclopropane-1-carboxylate synthase 2 (GRMZM2G164405) and two genes encoding ethylene-responsive transcription factors (GRMZM2G171569, GRMZM2G131281) showed decreased transcript abundance

Table 2 Effect of *P. putida* strain FBKV2 (DS + PP) inoculation on the expression of aquaporin genes in maize seedlings under drought stress

Maize ID	Annotation	log2-fold change
Aquaporins		
GRMZM2G392975	PIP1; 3; plasma membrane integral protein 1–3	− 1.0173
GRMZM2G014914	PIP2; 1; plasma membrane intrinsic protein 2	− 1.0668
GRMZM2G092125	PIP2; 2; plasma membrane intrinsic protein 2	− 1.6384
GRMZM2G047368	PIP2; 6; plasma membrane intrinsic protein 2	− 2.8169
GRMZM2G154628	PIP2; 4; plasma membrane intrinsic protein 2	+ 2.1657
GRMZM2G081843	PIP1; 5; plasma membrane intrinsic protein 1	− 1.2974
GRMZM2G108273	TIP4; 2; plasma membrane intrinsic protein 4	− 2.8111
GRMZM2G126582	NIP; plasma membrane intrinsic protein	− 2.4043
GRMZM2G103214	NIP1; 1; plasma membrane intrinsic protein 1.1	− 5.6032

− stands for downregulation, and + stands for upregulation

Table 3 Effects of *P. putida* strain FBKV2 (DS + PP) inoculation on the expression of abiotic stress-responsive genes in maize seedlings under drought stress

Maize ID	Annotation	log2-fold change
GRMZM2G077206	Glutathione *S*-transferase GST 27	+ 1.7438
GRMZM2G096269	Glutathione *S*-transferase12	+ 6.4303
GRMZM2G363540	Glutathione *S*-transferase GST 26	+ 1.8451
GRMZM2G005710	Dehydroascorbate reductase—DHAR4	+ 2.2768
GRMZM2G169890	Superoxide dismutase 4 Cu-Zn family	− 1.8406
GRMZM2G025992	Superoxide dismutase 2 Cu-Zn family	− 0.8772
GRMZM2G058522	Superoxide dismutase 4AP Cu-Zn family	− 1.3508
GRMZM2G088212	Catalase isoenzyme B	− 1.3917
GRMZM2G144153	Putative glutathione peroxidase	− 1.0804
GRMZM2G122296	Putative phosphoethanolamine *N*-methyltransferase (PEAMT)	+ 1.3067
GRMZM2G150248	Polyamine oxidase	+ 1.721
GRMZM2G042253	Chaperonin Cpn60	+ 2.1856
GRMZM2G049373	HSP40/DnaJ peptide	+ 2.0118
GRMZM2G074790	Chaperonin Cpn60	+ 1.1475
GRMZM2G091189	Chaperonin Cpn10	+ 1.1267
GRMZM2G137696	Heat shock protein 70 family	+ 1.2617
GRMZM2G429396	18.8 kDa class V heat shock protein	+ 1.0646

− stands for downregulation, and + stands for upregulation

in seedling colonized with *P. putida* strain FBKV2 (Table 4). The data suggest that genes encoding ABA and ET biosynthesis, signaling, and response are mostly downregulated in inoculated seedlings indicating a low level of drought stress.

The GO enrichment results revealed that genes involved in auxin signaling (*AUX/IAA7*) (GRMZM2G121309) and responses *GH3.8* (GRMZM2G053338), *GH3* (GRMZM2G378106), and *SAUR56* (GRMZM2G113135) showed decreased transcript abundance (Table 4) in *P. putida* strain FBKV2-inoculated seedlings, whereas five genes encoding late embryogenesis-abundant (LEA) (GRMZM2G014419, GRMZM2G017991, GRMZM2G087094, GRMZM2G331027, and GRMZM2G358540) proteins showed increased transcript abundance. The results suggest that the downregulation of auxins and upregulation of LEA proteins in FBKV2-inoculated maize seedlings could contribute to a higher level of drought tolerance.

Microbial signaling-associated genes

Two genes encoding indolin-2-one monooxygenase (*CYP71C2*) and indole-2-monooxygenase (*CYP71C4*) (GRMZM2G167549, GRMZM2G085661) showed increased transcript abundance in maize seedlings inoculated with strain FBKV2. *CYP71C2* and *CYP71C4* are involved in the biosynthesis of plant heteroaromatic metabolite benzoxazinoids (BXs) (Dutartre et al. 2012) which recruit *P. putida* (Neal et al. 2012).

Quantitative real-time-PCR validation of differentially expressed genes from RNA Seq

To investigate the accuracy of Illumina RNA Seq, 18 DEGs from each GO category were randomly selected from RNA Seq data and were analyzed by quantitative real time PCR (qRT-PCR). Correlation between RNA Seq and real-time PCR was evaluated using log2-fold change measurements. The results showed that the expression pattern of the 18 DEGs was significantly similar ($R^2 = 0.985$) and confirms the accuracy of the Illumina RNA Seq (Fig. 5).

Discussion

Drought stress affects almost every developmental stage of maize plants. However, phenophases such as germination, seedlings, and flowering are the major traits for crop establishment and are more prone to drought stress (Delachiave and Pinho 2003; Ashagre et al. 2014; Khayatnezhad et al. 2010; Aslam et al. 2015). Although several reports have come on microorganism-mediated drought tolerance at seedling stages (Grover et al. 2013; Sandhya et al. 2009) and our previous studies demonstrated that inoculation of maize seedlings with *P. putida* strain FBKV2 improved plant growth and resilience to drought tolerance (Vurukonda et al. 2016), little is known at molecular level. The present study was aimed to determine the potential of *P. putida* strain FBKV2 in the regulation of maize genes under drought stress condition.

Table 4 Effect of *P. putida* strain FBKV2 (DS + PP) inoculation on the expression of genes associated with hormonal signaling in maize seedling under drought stress

Maize ID	Annotation	log2-fold change
Response to abscisic acid		
GRMZM2G154987	Pyrabactin resistance-like protein 3	− 1.316
GRMZM2G479760	bZIP transcription factor ABI5	− 1.5596
GRMZM2G134628	2C-type protein phosphatase protein	− 2.4113
GRMZM2G059453	Protein phosphatase 2C (PP2C)-like	− 1.6011
GRMZM5G818101	Putative protein phosphatase 2C family protein	− 2.2982
GRMZM2G001243	Probable protein phosphatase 2C 50	− 2.3602
GRMZM2G300125	Protein phosphatase 2C ABI1	− 3.18
GRMZM2G159811	Probable protein phosphatase 2C 37	− 2.8445
GRMZM2G082487	Probable protein phosphatase 2C 68	− 2.137
GRMZM2G177386	2C-type protein phosphatase protein	− 2.1076
GRMZM2G147051	Serine/threonine-protein kinase like	+ 5.8436
GRMZM2G147857	Serine/threonine-protein kinase like	+ 3.1624
GRMZM2G084806	Serine/threonine-protein kinase like	+ 2.7826
Response to ethylene		
GRMZM2G052422	1-aminocyclopropane-1-carboxylate oxidase 1—ACO35	− 1.1456
GRMZM2G164405	1-aminocyclopropane-1-carboxylate synthase2—ACS47	− 1.609
GRMZM2G171569	Ethylene-responsive transcription factor ABI4-like	− 2.3026
GRMZM2G131281	Ethylene-responsive transcription factor 11-like	− 2.8038
Auxin		
GRMZM2G053338	Indole-3-acetic acid-amido synthetase GH3.8	− 2.1105
GRMZM2G378106	Indole-3-acetic acid-amido synthetase GH3	− 1.1893
GRMZM2G113135	SAUR56-auxin-responsive SAUR family member	− 1.2015
GRMZM2G121309	IAA7-auxin-responsive Aux/IAA family member	− 1.7499
LEA proteins		
GRMZM2G014419	Late embryogenesis abundant protein, LEA-14	+ 1.1907
GRMZM2G017991	Late embryogenesis abundant protein, LEA-14	+ 7.668
GRMZM2G087094	Late embryogenesis abundant protein, LEA-14	+ 1.3958
GRMZM2G331027	Late embryogenesis abundant protein like	+ 2.3533
GRMZM2G358540	Late embryogenesis abundant protein like	+ 2.9985

− stands for downregulation, and + stands for upregulation

Growth promotional studies under drought stress

To understand the molecular responses of maize seedlings to drought stress, two different plant treatments were analyzed: (i) plants in association with *P. putida* strain FBKV2 and drought stress (DS + PP) and (ii) uninoculated plants and drought stress (DS). Our results clearly show that inoculation with *P. putida* strain FBKV2 significantly enhanced the root and shoot length, soil moisture, and relative water content of seedlings compared to uninoculated treatment. These results validate the findings of Vurukonda et al. (2016), Grover et al. (2013), and Sandhya et al. (2010), who observed that rhizobacteria inoculation enhanced the plant growth, soil moisture, and relative water content of maize and sorghum seedlings. Furthermore, seedlings inoculated with *P. putida* strain FBKV2 survived up to 10 days after exposure to drought stress, whereas uninoculated plants died completely at the end of the sixth day. These results indicate that maize seedlings colonized with *P. putida* strain FBKV2 become more tolerant to drought stress than uninoculated seedlings. Drought tolerance promoted by rhizobacterial inoculation was already reported. Inoculation of maize with PGPR *P. putida* GAP-P45 (Sandhya et al. 2010) and *Azospirillum lipoferum* (Bano et al. 2013) improved plant growth through the accumulation of free amino acids and soluble sugars compared to non-treated plants under drought stress. Inoculation with ACC deaminase producing *Bacillus licheniformis* K11 alleviated drought stress in pepper plants (Hui and Kim 2013). Similarly, soybean plants inoculated with rhizobacterium *P. putida* H-2–3 improved plant growth performance under drought stress conditions (Sang-Mo et al. 2014).

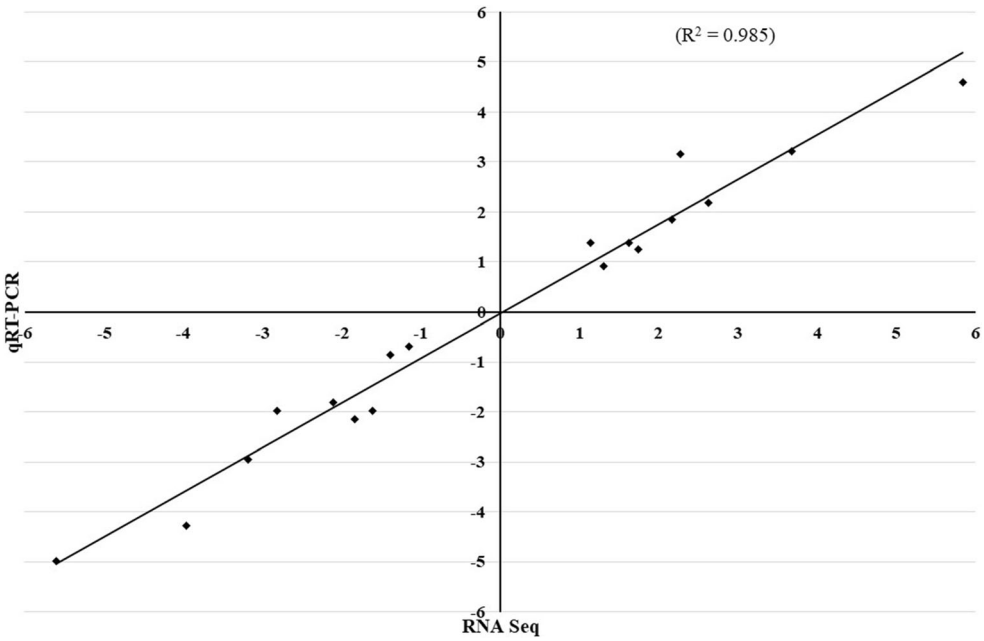

Fig. 5 Validation of RNA Seq using qRT-PCR. Correlation of fold change was analyzed by RNA Seq (*x*-axis) with data obtained from qRT-PCR (*y*-axis). Comparison of fold change was done using scatter plots generated using log2-fold change values obtained from RNA Seq and qRT-PCR. Dots represent the DEG.

Root colonization studies

Root colonization is the primary requirement to ensure an intimate association with the plant and thus supports rhizobacterial efficacy against water stress (Marasco et al. 2012). To check the ability of *P. putida* strain FBKV2 to colonize the maize root system, root colonization studies were performed. The results showed that plants were highly colonized by *gfp*-labeled *P. putida* strain FBKV2 after 4 days of drought stress and induced resilience to drought tolerance. Colonization of *P. putida* and *Bacillus amyloliquefaciens* in chickpea rhizosphere has been visualized using *gfp* labeling, and the synergistic effect of both the strains mitigated drought stress in chickpea (Kumar et al. 2016). Sultana et al. (2016) demonstrated successful root colonization of sorghum with *gfp*-labeled *P. putida* and *Azotobacter chroococcum* which enhanced nutrient uptake in sorghum. Furthermore, Fan et al. (2012) demonstrated colonization capability of *gfp*-labeled *B. amyloliquefaciens* in *Zea mays*, *Arabidopsis thaliana*, and *Lemna minor* under desert ecosystem.

RNA Seq

In the present study, we employed Illumina HiSeq 2500 sequencing technology to profile the transcriptome changes in the leaf tissues of inoculated and uninoculated maize seedlings under drought stress. A total number of 3738 DEG were identified in the maize seedlings inoculated with *P. putida* strain FBKV2. Among 3738 DEG, 1163 genes showed increased transcript abundance and 2575 genes showed decreased transcript abundance.

Response to carbohydrate metabolism

Carbohydrate metabolism is the major metabolism, which provides the essential saccharides and energy that the plant needs. The changes in the expression of genes involved in carbohydrate metabolism may induce the regulation that plants undergo during drought stress (Min et al. 2016). In our study, genes encoding starch biosynthesis and degradation showed decreased transcript abundance in *P. putida* strain FBKV2-inoculated seedlings; however, α-amylase and starch branching enzyme-III showed increased transcript abundance. The breakdown of starch in plants is catalyzed by amylase and with the interactive action of debranching enzyme, the stored starch breakdown into small oligosaccharides which provides energy to the plants under drought stress (Kaplan and Guy 2004).

The increase in the expression levels of the two *Suc synthases* encoding enzymes in FBKV2-inoculated seedlings suggests the possibility of sucrose synthesis which may help in osmotic adjustment and maintain homeostasis, allowing the plant to perform its normal cellular functions under drought stress (Krasensky and Jonak 2012), and sucrose hydrolysis results in the accumulation of glucose and fructose which serves as a starting point for glycolysis and synthesis of other saccharides. Our results validate the findings of Gagné-Bourque et al. (2016), who demonstrated that inoculation of timothy grass with *Bacillus subtilis* strain B26 increased sucrose concentrations in shoots under both non-stress and drought-stress conditions. Furthermore, inoculation with symbiotic *Neotyphodium coenophialum* in tall fescue plants alleviated drought stress, due to rapid accumulation of compatible

solutes such as sucrose, glucose, and fructose (Nagabhyru et al. 2013).

Response to membrane transporters

Aquaporins are membrane channel proteins which facilitate the transport of water across membranes in living organisms (Maurel et al. 2008). Few aquaporins can also facilitate the transport of other essential molecules such as CO_2, silicon, boron, urea, or ammonia (Li et al. 2014). Plant aquaporins belong to the gene family of major intrinsic proteins (MIPs), which is subdivided into five subfamilies based on amino acid sequence similarity: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and uncharacterized intrinsic proteins (XIPs), each group being also divided into several groups (Chaumont et al. 2001; Maurel et al. 2008; Gupta and Sankararamakrishnan 2009; Marulanda et al. 2010). Among the MIPs, PIPs are the major determinant in regulating water uptake by plants (Siefritz et al. 2002; Javot et al. 2003; Postaire et al. 2010). In the last few years, much effort has been concentrated on investigating the function and regulation of aquaporins upon inoculation with bacteria and fungi. Porcel et al. (2006) found a reduction in the expression of a plasma membrane aquaporin gene in the roots of soybean plants inoculated with the nitrogen-fixing bacteria *Bradyrhizobium japonicum* under both well water and water-deprived conditions. In our study, *P. putida* strain FBKV2 inoculation downregulated the expression of gene-encoding *PIP1; 3* and the results are in agreement with recent findings by Quiroga et al. (2017) who demonstrated that inoculation of drought sensitive and tolerant cultivars of maize with Arbuscular mycorrhizal fungi (AMF) downregulated the expression of *ZmPIP1; 3* gene. Similarly, Bárzana et al. (2014) reported that AMF inoculation downregulated *ZmPIP1; 3* and upregulated the expression of *ZmPIP2; 4* gene in maize leaf and the same gene was confirmed by Armada et al. (2015) in root tissue under drought stress. Furthermore, two genes encoding *ZmPIP2; 2* and *ZmPIP2; 4* were upregulated in AMF inoculated drought tolerant cultivars (Quiroga et al. 2017). In our study, gene-encoding *PIP2; 4* showed increased transcript abundance, whereas *PIP1; 5*, *PIP2; 1*, *PIP2; 2*, and *PIP2; 6* showed decreased transcript abundance. Arbuscular mycorrhizal symbiosis with maize plants has been shown to regulate aquaporin expression differentially in drought-tolerant and drought-sensitive maize cultivars especially *ZmTIP1; 1*, *ZmTIP2; 3*, and *NIP2; 1* that showed downregulation in both drought tolerant and sensitive cultivars, whereas *TIP4; 1* showed upregulation in both the cultivars under drought stress (Quiroga et al. 2017). In our study, *NIP*, *NIP1; 1*, and *TIP4; 2* showed decrease transcript abundance.

These results suggest that inoculation with *P. putida* strain FBKV2 downregulated aquaporins which might be minimized water flow through cell membranes and uphold leaf turgor thereby helping the seedlings from being affected by drought stress compared to the uninoculated seedlings.

Response to abiotic stress

Exposure of plants to drought stress leads to the generation of reactive oxygen species (ROS), including superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), singlet oxygen (O_{12}), and alkoxy radicals (RO). ROS react with proteins, lipids, and deoxyribonucleic acid causing oxidative damage and impede the normal functions of the plant (Vurukonda et al. 2016). In order to overcome the oxidative stress, plants develop antioxidant defense systems comprising both enzymatic and non-enzymatic antioxidants which prevents ROS accumulation and alleviate the oxidative damage (Miller et al. 2010; Vurukonda et al. 2016). Enzymatic antioxidants are ascorbate peroxidase (*APX*), catalase (*CAT*), glutathione peroxidase (*GPX*), superoxide dismutase (*SOD*), and peroxiredoxin (*PrxR*). Non-enzymatic components contain ascorbate and glutathione.

In the present study, the activity of enzymatic antioxidant *SOD*, *CAT*, and *GPX* in *P. putida* strain FBKV2-inoculated seedlings was significantly reduced as compared to uninoculated seedlings growing under drought stress. Our results are consistent with those reported by Naseem and Bano (2014), Vardharajula et al. (2011), and Sandhya et al. (2010) for maize where exopolysaccharide producing *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2), and *Alcaligenes faecalis* (AF3), as well as *Bacillus* and *Pseudomonas* species inoculation reduced the *CAT* and *GPX* activity under drought stress. Recently, Bulegon et al. (2016) and Saeed et al. (2016) reported that inoculation of *Urochloa ruziziensis* and *Brassica napus* with *Azospirillum* species decreased *SOD* activity under drought stress.

Glutathione *S*-transferases (*GST*) are important phase II, GSH-dependent ROS-scavenging enzymes found in the plants. *GST* catalyzes the conjugation of GSH to electrophilic sites on a wide range of phytotoxic substrates (Labudda and Safiul Azam 2014). Inoculation of strawberries with *P. polymyxa* RC05, *P. fluorescens* RC77, *P. fluorescens* RC86, *P. putida* RC06, *P. putida* 29/2, and *Rhodococcus erythropolis* RC9 enhanced the activity of *GST* compared to uninoculated control under drought stress. Similarly, under non-stress condition, *Arabidopsis thaliana* inoculated with *Piriformospora indica* upregulated two proteins with homology to GST (Pesškan Berghöfer et al. 2004). In our study, gene-encoding glutathione *S*-transferases—12, 26, and 27—showed increased transcript abundance in *P. putida* strain FBKV2-inoculated seedlings compared to uninoculated treatment.

Dehydroascorbate reductase (*DHAR*) catalyzes the reduction of dehydroascorbate (*DHA*) to ascorbic acid (Asc) and

provides protection against oxidative damage in plants (Chen and Gallie, 2006). Recently, Kim et al. (2014) reported that overexpression of a rice cytosolic DHAR gene conferred enhanced salt stress tolerance to rice plants by maintaining the *Asc* pool (Kim et al. 2014). Similarly, transgenic tobacco overexpressing *Arabidopsis* cytosolic DHAR showed enhanced tolerance to ozone and drought stresses (Eltayeb et al. 2006). Our results are in agreement with the findings of Hasna et al. (2016) who reported that PGPR inoculated okra plants showed higher expression of *DHAR* than uninoculated control plants and confer salinity tolerance in okra. In our study, gene-encoding *DHAR4* showed increased transcript abundance in maize seedlings inoculated with strain FBKV2.

Plants possess a number of adaptive mechanisms to deal with drought stress to maintain tissue turgor pressure (Ashraf 2010; Gou et al. 2015). One of the most efficient mechanisms is osmotic regulation through the accumulation of water-soluble compounds known as "compatible solutes" that enable plants to cope with drought stress (Ashraf and Foolad 2007; Ashraf 2010; Gou et al. 2015). Among the compatible solutes, glycine betaine (*GlyBet*) and choline (*Cho*) are the important osmoprotectants that confer tolerance to abiotic stresses in plants (Rhodes and Hanson 1993; Gorham, 1995; Sakamoto and Murata 2000; Zhang et al. 2010). Choline plays a critical role in plant stress resistance, mainly for enhancing *GlyBet* synthesis and accumulation (Zeisel 2006; Zhang et al. 2010). Plant *Cho* is synthesized in the cytosol via stepwise methylation of ethanolamine derivatives (Zeisel and Blusztajn 1994). The cytosolic enzyme phosphoethanolamine *N*-methyltransferase (*PEAMT*) catalyzes the methylation steps in the *Cho* biosynthetic pathway (Nuccio et al. 1998, 2000; Zhang et al. 2010). Evident reports on the induced role of *Bacillus subtilis* GB03 in *Arabidopsis* showed obvious enhancements in biosynthesis and accumulation of choline as a precursor in GB metabolism (Zhang et al. 2010). The relative expression of *PEAMT* gene induced in *Arabidopsis* by *Bacillus subtilis* GB03 was almost 3-fold as compared with uninoculated plants under osmotic stress, resulting in an elevated metabolic level of choline together with GB in osmotically stressed plants (Zhang et al. 2010). Furthermore, inoculation of maize with PGPR strains *Klebsiella variicola* F2, *P. fluorescens* YX2, and *Raoultella planticola* YL2 induced higher accumulation of choline and GB than those plants without inoculation under different degrees of drought stress (Gou et al. 2015). In our RNA Seq experiment, gene-encoding putative phosphoethanolamine *N*-methyltransferase (*PEAMT*) was upregulated in *P. putida* strain FBKV2-inoculated seedlings compared to uninoculated treatment. These observations, in the light of available literature, suggest the induced role of *P. putida* strain FBKV2 in conferring drought tolerance by expressing *PEAMT* in maize seedlings.

Drought stress induce the accumulation of osmolytes such as polyamines (Zhou et al. 2017; Sperdouli and Moustakas 2012; Groppa and Benavides 2008). Polyamines (PAs), widely present in living organisms, are now regarded as a new class of growth substances which includes spermidine (*Spd*, a triamine), spermine (*Spm*, a tetramine), and their precursor putrescine (*Put*, a diamine) which play an important role in the regulation of plant developmental and physiological processes (Kusano et al. 2007; Gill and Tuteja 2010). Furthermore, accumulation of cellular PAs significantly enhances the tolerance of plants to various abiotic stresses due to the upregulation of stress-related genes (Zhou et al. 2017, 2015; Wen et al. 2008). In our study, gene-encoding polyamine oxidase responsible for the conversion of spermine to spermidine showed increased transcript abundance in *P. putida* strain FBKV2-inoculated seedlings. Zhou et al. (2017) investigated the effects of spermidine producing *Bacillus megaterium* BOFC15 on *Arabidopsis* plants under drought stress. Inoculation with BOFC15 enhanced primary and lateral roots than the control plants. Furthermore, the cellular *Spd* and *Spm* contents were higher in the BOFC15-inoculated plants and displayed stronger ability to tolerate drought stress than control plants. Our data suggest that *P. putida* strain FBKV2 inoculation upregulated the expression of polyamine oxidase that resulted in the accumulation of spermidine, conferring drought tolerance in maize seedlings.

Protein dysfunction is a common consequence of abiotic stresses. Plants respond to abiotic stresses, through a number of mechanisms to maintain their proteins in a functional form. Several proteins such as heat shock proteins (HSPs) are synthesized by the plants, and these proteins are responsible for protein folding, localization, accumulation, and degradation and thus are believed to play an important role in cellular processes and impart tolerance to abiotic stresses (Feder and Hofmann 1999; Wang et al. 2004; Wahid et al. 2007). In the present study, six HSPs (*Cpn60*, *Cpn10*, *Hsp40*, *Hsp70*, and *Hsp18.8* class V) showed increased transcript abundance in FBKV2-inoculated seedlings compared to uninoculated control. These results validate the findings of Lim and Kim (2013), who observed a higher level of expression of sHSP in pepper plants inoculated with *Bacillus licheniformis* K11 under drought stress. Similarly, priming of wheat seedlings with *A. brasilense* NO40 significantly alleviated the deleterious effect of drought stress by upregulating the gene-encoding *Hsp*17.8 (Kasim et al. 2013).

Response to hormonal signaling

Abscisic acid (ABA) is the best-known plant hormone in conferring tolerance to abiotic stresses including drought and salinity (Wasilewska et al. 2008; Llanes et al. 2016). Three protein classes such as pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptor (*PYR/PYL/RCARs*) proposed to be the ABA receptors, *protein phosphatase 2Cs* (*PP2Cs*) which act as negative regulators, and

SNF1-related protein kinase 2 s (*SnRKs*) which are positive regulators (Mustilli et al. 2002; Park et al. 2009; Schweighofer et al. 2004; Umezawa et al. 2009; Yoshida et al. 2006) are involved in ABA signal transduction pathways. In the present study, strain FBKV2 inoculation decreased transcript abundance of major key regulators such as *PYL3* and *PP2C* isoforms such as *PP2C*, *2C 50*, *2C ABI1*, *37*, and *68*. Our results are inconsistent with that of Vargas et al. (2014) who reported the downregulation of PYL8, PP2C, and *SnRK2* in sugarcane inoculated with *Gluconacetobacter diazotrophicus* PAL5, whereas in our study, FBKV2 inoculation enhanced *SnRK2* family proteins such as plant-specific *Ser/Thr kinases*. In the presence of ABA, the *PYR/PYL/RCAR* forms a complex with *PP2C*, allowing the activation of *SnRKs* which target ion channels, membrane proteins, and transcription factors and facilitate transcription of ABA-responsive genes, thereby conferring drought tolerance.

In the biosynthetic pathway of ethylene, *S*-adenosylmethionine (S-AdoMet) is converted by 1-aminocyclopropane-1-carboxylate synthase (*ACS*) to 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene (Grover et al. 2011). Under both biotic and abiotic stress conditions, the plant hormone ethylene endogenously regulates homeostasis of plants resulting in reduced growth. Plant ACC is sequestered and degraded by ACC deaminase producing bacteria to supply nitrogen and energy (Vurukonda et al. 2016). Furthermore, by removing ACC, the bacteria reduce the deleterious effect of ethylene, ameliorating plant stress and promoting plant growth (Glick 2005). Several reports showed the role of ACC deaminase producing bacteria in mitigating drought stress in crops (Mayak et al. 2004; Dodd et al. 2005; Zahir et al. 2008; Shakir et al. 2012; Sharma et al. 2013; Hui and Kim 2013). In the present study, ACC deaminase producing *P. putida* strain FBKV2 inoculation lowers the expression of ethylene biosynthesis genes *ACO35* and *ACS47*. Our results are in agreement with Vargas et al. (2014) who reported that inoculation of sugarcane with *G. diazotrophicus* lowers the expression of *ACO* and *ACS6* genes.

Auxin plays an important role in all the aspects of plant growth and development. Among the most commonly studied auxin signaling gene families are *SAURs*, *GH3s*, and *Aux/IAAs*. Auxin has already been reported as a negative regulator of drought tolerance. In wheat, drought stress tolerance was accompanied by a decrease in IAA content (Xie et al. 2003; Vargas et al. 2014). In the present study, FBKV2 inoculation decreased transcript abundance of genes encoding *GH3*, *GH3.8*, *SAUR56*, and *AUX/IAA* in maize seedlings under drought stress. Similar results were reported by Vargas et al. (2014) in sugarcane roots inoculated with *G. diazotrophicus* PAL5 under drought stress.

Downregulation of IAA was found to facilitate the accumulation of late embryogenesis-abundant (*LEA*) mRNA, leading to drought stress adaptation in plants (Zhang et al. 2009; Vargas et al. 2014). In our studies, FBKV2 inoculation increased the transcript abundance of genes encoding *LEA* proteins. The results suggest that the downregulation of auxin signaling and response pathway and accumulation of *LEA* proteins could contribute to drought tolerance in maize seedlings inoculated *P. putida* strain FBKV2.

Plant-microbe signaling-associated genes

Benzoxazinoids (BXs), such as 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA), are heteroaromatic metabolites with benzoic acid moieties which are produced during relatively early, vulnerable plant growth stages (Neal et al. 2012). Exposure of *P. putida* KT2440 to DIMBOA induced bacterial genes with putative functions in chemotactic responses; furthermore, in vitro chemotaxis assays also proved that *P. putida* KT2440 displayed taxis towards DIMBOA (Neal et al. 2012). In our study, two genes encoding *CYP71C2* and *CYP71C4* showed increased transcript abundance in maize seedlings inoculated with strain FBKV2. The upregulation of *CYP71C2* and *CYP71C4* may enhance the biosynthesis of BXs and attracted *P. putida* strain FBKV2 and resulted in root colonization there by imparting drought tolerance in maize seedlings.

Conclusions

Summarizing our data, the major outcome of this study is that the *P. putida* strain FBKV2 inoculation triggered drought tolerance in early stages of maize seedlings. By studying the leaf transcriptome using RNA Seq technology, we could provide data on transcripts for the major genes involved in carbohydrate, membrane transporters, detoxification, and hormonal signaling and discussed their potential role in maize drought tolerance. The data from our study can be utilized to better understand the gene expression networks involved in the other plant-microbe interaction under drought stress and could provide tools to maximize the benefits for crop production.

Author contributions SkZ and S.V designed the experiments. SkZ and S.S.K.P.V optimized and performed the experiments. SkZ performed the data analysis. SkZ and S.V wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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