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Microbacterium oxydans Regulates Physio-Hormonal and Molecular Attributes of *Solanum lycopersicum* under Drought Stress

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Abstract: Among the types of abiotic stresses, drought is one of the most serious growth limiting factors for crop plants. In recent years, various strategies have been employed to alleviate the adverse effects of drought stress in crops, but the use of plant growth-promoting rhizobacteria (PGPR) is among the standout techniques. Currently, we have isolated 55 strains of bacteria from the rhizosphere of *Achyranthes aspera* L. and *Calotropis procera* (Aiton). However, AGH3, AGH5, and AGH9 produced significant ($p = 0.05$) amounts of plant hormones and exhibited siderophore and phosphate solubilization activities. Bioassay experiments on *Waito*-C rice demonstrated an enhanced growth in the presence of the isolate AGH3. Moreover, the isolate AGH3 promoted the growth of *Solynum lycopersicum* L. under drought stress. The results revealed that AGH3-associated *S. lycopersicum* plants showed significantly ($p = 0.05$) reduced production of abscisic acid (ABA) and jasmonic acid (JA) as compared with the AGH3-free plants under polyethylene glycol (PEG) stress. In addition, high expressions of *SlmiR* 159 (from 6- to 10-fold), *SlHsfA1a* (from 1- to 4-fold), and *SlHAKT1* (from 0.26- to 1-fold) genes were noticed in AGH3-associated *S. lycopersicum* plants under drought stress. From the results, it is concluded that rhizobacteria (AGH3) can be used as a pragmatic biofertilizer to ensure organic farming under normal as well as drought conditions.

Keywords: crop plants; rhizobacteria; drought stress; ABA; IAA; JA; gene expression

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1. Introduction

Global population growth and climate change pose challenges to crop production all over the world. Crops are consistently exposed to numerous ecological challenges, such as high heat and scarcity of water, elevated saline conditions, waterlogging, heavy metals, UV radiation, and a lack of readily available soil nutrients. These ecological challenges affect plant growth and development through impaired plant physicochemical attributes. In addition, they also damage the soil environment, which ultimately reduces crop productivity [1–4]. According to the estimates, 70% of major crop yields and 90% of arable land in the world are lost due to environmental stresses [5,6]. According to Suzuki et al. [7], the United States of America (USA) experienced a loss of USD 200 billion as a result of drought and heat stress. Plants regulate the molecular mechanisms that are directly linked to plant growth and productivity, but abiotic stresses can interrupt such

mechanisms [5,8–11]. According to Fedoroff et al. [12,13], plant-associated microorganisms can influence plant reactions to various environmental factors, including drought stress. This is one of the reasons that plant–microbe interactions have received significant attention in recent years. The associations of microorganisms with host plants are essential to sustain agriculture [14,15]. The soil microbiome is home to millions of species, including fungi, bacteria, viruses, archaea, and protozoa. The rhizospheric microbial flora are one of the stress relievers that can affect the microbial diversity in soil and can improve plant growth.

Drought stress affects the normal biochemical, physiological, and morphological processes in plants. Generally, it reduces leaf size, stem extension, root proliferation, photosynthesis, nutrient uptake, and water retention in plant species [16]. Among other biochemical and physiological processes of plants, drought stress mainly affects chloroplast function and stomatal regulation [17]. It is widely recognized that abiotic stress triggers the interaction of signal transduction cascades with that of phytohormones and defense antioxidants [18]. In addition, changes in the stress-responsive hormones play a central role in the regulation of plant growth under stress [19]. During drought stress, the levels of ABA increase via ABA biosynthesis to overcome the stress [20,21]. ABA is a primary phytohormone responsible for opening and closing stomata and regulating root growth by optimizing water uptake during drought stress [18]. Similarly, JA is another phytohormone that plays an important role in alleviating drought stress. JA stimulates the anti-oxidative system to protect plants from oxidative stress and ROS generation. Additionally, alterations in gene expressions protect plant species from the deleterious effects of drought. Such genes regulate heat shock proteins (HSPs), heat shock transcription factors (HSFs), stress-induced proteins, and stress proteins that induce stress tolerance in plants. Heat-shock transcription factors (HSFs) are found in almost all organisms, and they regulate the genes encoding molecular chaperones and other stress proteins required for survival on the inception of acute stress [22]. Similarly, Wang et al. [23] reported that drought stress induced the expression of transcription factor HsfA1a to promote tolerance in plants against drought stress. On the other hand, HKT1 has been reported to promote higher concentrations of cellular K during stress to encourage normal functioning of the plant [24]. Ali et al. [25], Fairbairn et al. [26], and Almeida et al. [24] reported that higher expression of the SlHKT1 gene stimulated high K production. Indeed, the accumulation of K contents plays an important role in the biochemical and physiological processes of plant growth, as well as the survival of plants under stress [23]. Under drought stress, the diffusion rate of K in soil toward the roots decreases, depressing plant resistance to drought stress [20,23].

Drought in any form can significantly affect global food production and agricultural systems [3,27,28]. Given the current state of climate change and the expanding human population, water availability is a major challenge for agriculture. A total of 40% of the world's food is produced through irrigation, which uses 2% of the earth's arable land area [29]. Irrigated farmland typically yields at least twice as much produce per square meter as rain-fed agriculture [30]. Approximately from 50 to 90% of the fresh weight of plants is made up of water [31,32], with higher amounts found inside the cells and the remaining in cell walls [33]. One of the biggest challenges in modern agriculture is to sustain growth and crop production during biotic and abiotic stresses, including drought [34]. Due to the ongoing rise in temperature and water scarcity, severe drought conditions are occurring more frequently. The global cereal production decreased by 9–10% as a result of drought, which significantly restrainted food supply in the world market. Under drought conditions, from 30 to 90% of the yield can be lost depending on the crop species, as some species are more vulnerable to drought stress than others [35]. In China, drought stress decreased corn yield by more than 30% [36], maize yield by 40%, and wheat yield by 21% [37]. Similarly, the loss in cowpea production has been reported to be 34–68%, which was mainly dependent on the time that the drought occurred [38]. In the last few decades, attempts have been made to mitigate the negative impacts of abiotic stressors on crops. Cutting-edge irrigation and agricultural practices are some of the ways

that have made significant contributions in regards to crop yield [5,39], but these methods have also negatively affected the environment. For example, the majority of fertilizers and chemicals that are typically used in agricultural fields can affect the populations of beneficial soil microbes. About 0.1% of insecticides and pesticides is thought to have an effect on the intended organisms, while the remainder contaminates the immediate environment [13,40]. Additionally, the development of drought-tolerant cultivars has eased the detrimental impacts of drought stress on crops; however, this method is labor intensive and time-consuming. Additionally, the marketability of transgenic crops cannot be guaranteed because of consumer acceptance concerning genetically modified crops [13]. Moreover, a number of plant growth-promoting microorganisms have been reported to enhance crop productivity under abiotic stresses [41]. Plant growth-promoting bacteria (PGPB) have positive impacts on a variety of plants and are crucial for boosting plant growth, biological control, and stress resistance [13,40,42–44]. Plant development is promoted by PGPB through both direct and indirect mechanisms in a range of settings [45]. Direct processes that have been discovered, so far, include the production or regulation of phytoregulators; the release of volatile chemicals that influence plant signaling pathways; and the improvement of nitrogen, phosphorus, and iron uptake by plants [13,46–50]. PGPR from different genera, including *Bacillus, Enterobacter, Erwinia, Escherichia, Flavobacterium, Pseudomonas*, and *Serratia* have been reported for their roles in drought stress tolerance in different crop plants [51,52].

Tomato is a key vegetable crop throughout the world. Tomatoes are delicious, and they are also a very beneficial source of vitamins A and C. Additionally, tomatoes are a model plant with theoretical and practical value in research. In Pakistan, tomatoes are produced on 24,144 hectares of land, which results in 2,75,241 tons of tomato production annually. The 4230 hectare Khyber Pakhtunkhwa (KP) region is crucial arable land that can produce annually around 51,062 tons of agricultural products. In addition, the 944 acres of land in the valley of Peshawar is dedicated to tomato cultivation that produces 2.978 thousand tons a year. Tomatoes need enough water to grow since they are susceptible to drought stress. To overcome this problem, in this study, we used drought-resistant microorganisms in tomato plants to ensure their normal growth under drought. For this purpose, we isolated rhizobacteria and tested their plant growth-promoting traits such as IAA production, siderophore synthesis, phosphate solubilization, and PEG stress tolerance. Moreover, the selected rhizobacteria were used as bioinoculants to test their effects on tomato plants under normal and PEG-stressed conditions.

2. Materials and Methods

2.1. Sample Collection and PGPR Isolation

We isolated bacteria from the rhizosphere of *Achyranthes aspera* L. and *Calotropis procer* from the dry, yet inhabited, waste land on the banks of the Indus River in District Swabi, Pakistan (latitude and longitude coordinates are 34.120155 and 72.470154, respectively). These plants are both famous weeds and have been used for medicinal purposes for a long time. The plants were identified in the taxonomy laboratory, Department of Botany, Abdul Wali Khan University Mardan. Samples of rhizosphere soil were separately filled in sterile zip lock bags and maintained at 0–6 °C until further use. The rhizosphere soil (1 g) obtained from the vicinity of the selected plant roots was serially diluted with saline water (0.85%). Subsequently, morphologically different colonies were formed by spreading of successive dilution series on LB growth medium and incubating at 28 °C until colonies formed. Through streaks on a Luria-Bertani (LB) agar plate, the bacteria were purified. The pure colonies were stored in 75% glycerol stock until further use. After 24 h of incubation, distinct traits including color, shape, size, and growth pattern of the colonies were assessed to identify different bacteria species.

2.2. Growth Promotion of Waito-C Rice under Drought Stress

Based on multi-PGP traits, we selected isolates that were screened on *Waito-C* rice (GA deficient rice). Initially, the rice seeds were surface sterilized by dipping the seeds in 20 mL of ethanol (70%) for two minutes, and then in NaOCl (1%) for 5 min. Then, the seeds were washed with sterile distilled water three times to remove traces of ethanol and NaOCl. The sterilized rice seeds were treated with rhizospheric bacterial isolates (10^9 CFU/mL) for 24 h and kept in a shaking incubator. Then, the seeds were grown for 20 days on autoclaved filter paper under a controlled environment (14 h = light, 10 h = dark, temperature = 28 °C, relative humidity = from 60 to 70%, light intensity = 250 mol·m^{-2} s^{-1}). Additionally, we used LB medium and 0.1% of a cultured fraction was injected into 20 mL of sterilized LB media to test 5 different concentration doses of PEG-60000 (0, 5, 10, 15, and 20%). The absorption spectrum of the medium was determined at 600 nm, while it was maintained in a shaking incubator at 30 °C (T60 UV spectrophotometer, PG Instruments Ltd., United Kingdom).

2.3. Effect of Selected Isolate on Tomato Growth under PEG Stress

The tomato seeds were purchased from the seed program of the National Agriculture Research Centre (NARC), Islamabad. The seeds were surface decontaminated with 70% EtOH followed by 2.5% NaOH, and then rinsed with deionized dH_2O. The disinfected seeds were transferred to plastic trays filled with coco peat (45–50%), zeolite (6–8%), perlite (35–40%), peat moss (10–15%), NH_4^+ (0.09 mg g^{-1}), NO_3 (0.205 mg g^{-1}), K_2O (0.1 mg NO_3 (0.205 mg g^{-1}), and P_2O_5 (0.35 mg g^{-1}). The trays were placed in a culture room under set conditions (14 h day/night cycle at 27 °C and a 10 h night at 24 °C, relative humidity of 60–70%, and sodium lamp light intensity of 1000 mol.m^{-2} s^{-1}). Equally sized seedlings were chosen at the V1 stage and transplanted into plastic pots (24 × 17 cm) filled with the same soil as germination trays. There was one plant in each pot, with a total of 12 replicates for each treatment.

The study was comprised of the following 10 treatments: (A) tomato plants grown under control condition (water only), (B) tomato plants inoculated with AGH3, (C) plants treated with 5% PEG, (D) plants treated with 5% PEG stress and inoculated with AGH3, (E) plants treated with 10% PEG, (F) plants treated with 10% PEG stress and inoculated with AGH3, (G) plants treated with 15% PEG stress, (H) plants treated with 15% PEG stress and inoculated with AGH3, (I) plants treated with 20% PEG stress (J) plants treated with 20% PEG stress and inoculated with AGH3, according to the detailed methods of [32,52].

In the case of inoculated pots, 50 mL of freshly diluted bacterial culture (10^8 CFU) was added to each pot and the course of action was repeated at an interval of 5 days until 15 days. After 15 days, 50 mL of PEG (5%, 10%, and 15%) was applied to each pot for 7 days, except pot A. The seedlings were harvested after 2 weeks of treatment, and growth parameters were recorded (SPAD-502, Konica-Minolta Sensing, Osaka, Japan).

2.4. Levels of Endogenous Phytohormones in Tomato under PEG Stress

Briefly, 0.2 g of freeze-dried shoot samples were subjected to an HPLC system (Shimadzu) fitted with a C18 reverse phase column (HP Hypersil ODS, particle size 5 m, pore size 120, Waters) and a fluorescent detector (Shinadzu RF-10AxL) with excitation at 305 nm. The flow rate was maintained at 1 mL/min. In contrast, 0.5 g of powdered material was mixed with 30 mL of extracting buffer and 10 ng of the abscisic acid standard [(ABA)-3,5,5,7,7,7-d6] for the abscisic acid analysis.

For the GC-MS/SIM examination, extract was dried, and methylated with diazomethane (Table 1). By means of the Lab-Base (ThermoQuest, Manchester, UK) data scheme program, the monitor responses to the ions at m/z 190 and 162 for Me-ABA and 194 and 166 for Me-[2H6]-ABA were acquired.

Table 1. GC/MS–SIM settings for the estimation of the ABA and SA.

Machine	Mass Selective Detector (Hewlett-Packard 6890, 5973N)
Column	HP-1 capillary column (30 m × 0.25 mm i.d. 0.25 µm film thickness) (J & W Scientific Co., Folsom, CA, USA)
Carrier gas Head pressure	He 40 mL/min 30 kPa
Source temperature	250 °C
Oven conditions	ABA: 60 °C (1 min) → 15 °C/min → 200 °C →5 °C/min → 250 °C →10 °C/min → 280 °C JA: 60 °C (2 min) → 10 °C/min → 140 °C (3 min) →3 °C/min 170 °C → 15 °C/min → 285 °C (8 min)
Injector temperature	200 °C
Ionizing voltage	70 ev

2.5. Molecular Identification of the Bacterial Isolate AGH3

Genomic DNA was obtained from GAH3 (200 mL) by using an QIAmp DNA Minikit (Qiagen, Hilden, Germany), in accordance with the assembly specifications. The 16S rRNA gene-specific primers 27F primer (5′-AGAGTTTGATCACTGGCTCAG-3′) and 1492R primer (5′-CGGCTTACCTTGTTACGACTT-3′) were used to amplify the isolated DNA for PCR. The PCR reaction were carried out in a 50 µL reaction volume containing 2500 ng bovine serum albumin, 15 pmol of each primer, 5 mM of dNTPs 4 µL, 1.25 U of Ex Taq DNA polymerase (Takara Bio Inc., Ostu, Shiga, Japan), and 1 µL of template DNA (5–30 ng/µL). The 30-cycle amplifications included denaturation at 94 °C (60 s), annealing at 55 °C (30 s), extension at 72 °C (60 s), and termination at 72 °C. The PCR result was purified using a Wizard® PCR Preps DNA Purification System (Promega, Madison, WI, USA).

Following the assembly specifications, a Big Dye Terminator Cycle sequencing kit was used to directly sequence purified double-stranded PCR fragments. Sequences for each zone were changed using Chromas Lite 2.01, URL: http://www.technelysium.com.au/chromas.html (accessed on 12 February 2022). Nucleotide sequence homology was compared for the 16S region of bacteria using BLAST, URL: http://www.ncbi.nlm.nih.gov (accessed on 14 August 2022). The ClustalW and MEGA (6.0) softwares were used to align the sequences in order to create a neighbor tree. The nodes in the phylogenetic trees were also supported statistically by the use of bootstrap replication (1000 replications).

2.6. RNA Extraction, cDNA Synthesis, and qRT-PCR Analysis for Gene Expression

We followed the modified protocol of Khan et al. (2020) for the RNA extraction. By means of 1 mL of Trizol® reagent (Invitrogen, USA) and 100 mg of smashed tomato leaves, total RNA was extracted, and then centrifuged at 12,000× *g* for 10 min at 4 °C. After being transferred to new vials (1.5 mL), the supernatant was mixed thoroughly for 15 s, chilled for 3 min, and then spun one more time (12,000× *g*, 15 min, 4 °C). The top layer was transferred to a new tube together with isoproponal and 1.2 M NaCl/0.8 M Na-citrate. The combination was kept at ambient conditions for one hour before being spun (12,000× *g*, 10 min, 4 °C).

Then, the residues were re-suspended in DEPC water after being rinsed using 1 mL 75% EtOH and spun once over (7500× g, 5 min at ambient conditions). The supernatant was removed. A qPCRBIO SYBER Green Kit was used to perform qRT-PCR utilizing synthesized (1 µL) gene-specific primers (Supplementary Table S1) and cDNAs as templates. The individual gene was normalized for corresponding response using actin, and the transcription level in control plants. A 20 µL volume containing 7 µL ddH_2O, 1 µL primer, 10 µL SYBER green, and 1 µL cDNA was used for the experiment. The following conditions were utilized with a total sample volume of 50 µL: initial fragmentation

by 94 °C for 5 min, 40 cycles of denaturation at 94 °C for 30 s, annealed at 58 °C for 30 s, extension at 72 °C for 1 min.

2.7. Statistical Analysis

All the experiments were run three times and the analysis of the data was performed using one-way ANOVA and the Dunnett post hoc test (significance level 0.05). Throughout the trial, all ten treatments were repeated three times. The effects of PGPR in controlling plant growth on rice germination were contrasted using a fully randomized approach. Graphpad Prism 5 was used to carry out the statistical analyses and the graphic display (Graphpad by Dotmatics, Boston, MA, United States of America).

3. Results

3.1. Screening of Selected PGPR on Waito-C Rice (GA Deficient Mutant)

Based on multiple PGP traits, such as IAA, siderophore, gibberellic acid, and phosphate solubilization activities, eleven isolates showed positive multi-PGP traits, while three strains performed outstandingly (Table 2).

Table 2. Multi-trait PGP characteristics of AGH3, AGH5, and AGH9.

Rhizobacteria	Phosphate Solubilization	IAA Production		GAs Production	Siderophore Production
		Positive/Negative with L. Tryptophan	Positive/Negative without L. Tryptophan		
AGH3	+++	+++	++	++	+++
AGH5	+	+	++	+	++
AGH9	++	++	+	+	+

+++ (High activity); ++ (Medium); + (Low)

The three isolates were further screened on *Waito-C* rice that exhibited enhanced rice growth as compared with the control; however, the AGH3-inoculated *Waito-C* rice plants showed higher growth (Figure 1 and Table 3). In addition, the PEG stress treatments revealed that the isolate AGH3 significantly ($p = 0.05$) tolerated the presence of 15% PEG in the media (Figure 2). The isolate AGH3 was further evaluated for different activities and subjected to molecular identification.

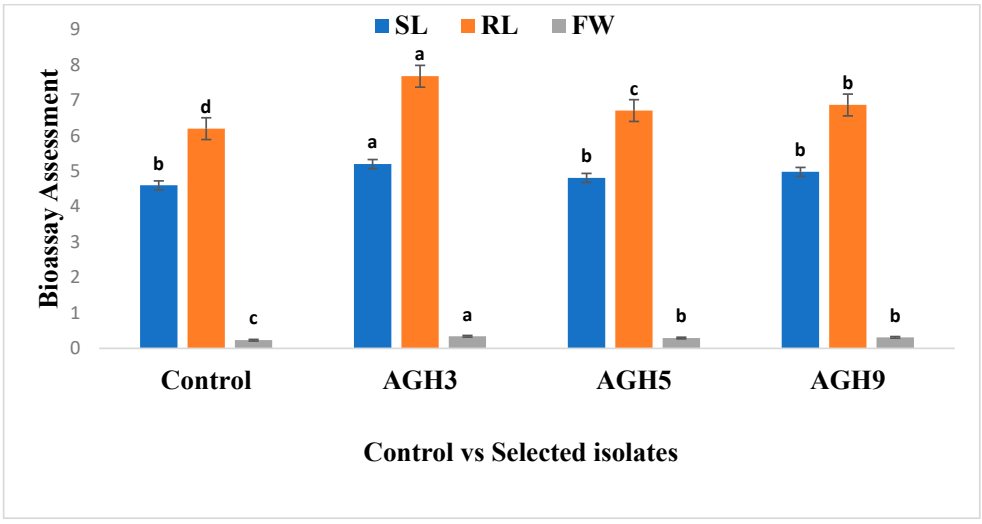

Figure 1. Bioassay assessment for growth promotion activity of selected isolates (AGH3, AGH5, and AGH9). Shoot length (SL); root length (RL); and fresh weight (FW). Different letters in the column show that values are significantly different ($p < 0.05$) from each other as evaluated from the DMRT test.

Table 3. Bioassay assessment for growth promotion activity of the competent isolate AGH3 in *Waito-C* rice. Different letters in the column show that values are significantly different ($p < 0.05$) from each other as evaluated from the DMRT test.

	Control Plant	AGH3 Treated Plant
Shoot Length (cm)	4.6 ± 1.0 b	5.2 ± 0.34 a
Root Length (cm)	6.2 ± 0.34 b	7.68 ± 1.0 a
Fresh Weight (g)	0.21 ± 0.25 b	0.37 ± 0.3 a

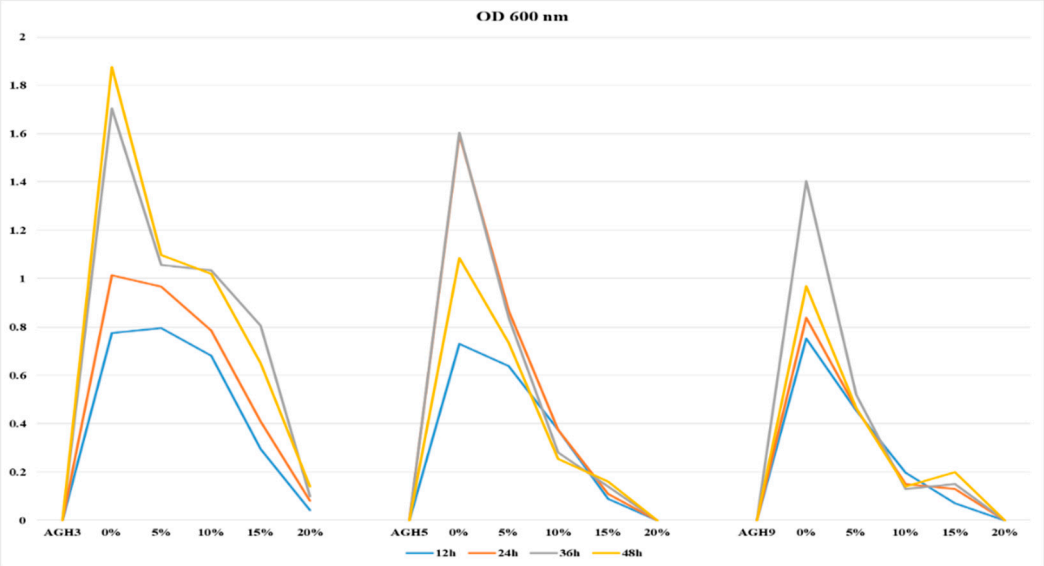

Figure 2. Screening of selected isolates AGH3, AGH5, and AGH9 for PEG tolerance.

3.2. Screening of Selected Isolates for PEG Tolerance

The selected isolates were tested for drought stress tolerance in LB medium (spiked with 0%, 5%, 10%, and 15%, and 20% PEG). Under different concentrations of PEG, the AGH3 isolates resisted the presence of PEG up to 20%, whereas AGH9 showed tolerance against 15% PEG (Figure 2).

The isolated strain AGH3 significantly ($p = 0.05$) boosted plant height and chlorophyll contents of the drought-stressed tomato plants, followed by AGH9 and AGH5 (Table 4). In fact, the tolerance of AGH3 to PEG was more consistent and it promoted the growth of tomato plants under PEG stress, therefore, it was selected for further experiments.

Table 4. Role of selected isolates on plant height and total chlorophyll contents of the *S. lycopersicum*.

	Plant Height (cm)					SPAD (Chlorophyll Meter)
	R1	R2	R3	R4	AVG	
Control (Normal)	43	46	49	51	47.25	44.1
Control (Drought)	25	25	33	30	28.25	26
AGH3	48	51	51	50	50	50
AGH5	46	45	47	45	45.85	44
AGH9	47	45	46	46	46	45

3.3. PGP Effect of AGH3 on Solanum lycopersicum L. under PEG Stress

The PEG stress unfavorably affected the agronomic features of *S. lycopersicum* (Table 5 and Figure 3). However, the association of AGH3 promoted tomato growth

and development under normal as well as varied PEG concentrations (5%, 10%, and 15%). Under normal conditions, AGH3-inoculated plants had significantly ($p = 0.05$) increased shoot lengths (26.66%), root lengths (17.24%), shoot and fresh weights (78% and 59.04%, respectively), shoot/root dry weights (65.69% and 48.71%, respectively), and chlorophyll contents (7.48%).

Table 5. Effect of AGH3 on PGP attributes. Different letters in the column show that values are significantly different ($p < 0.05$) from each other as evaluated from the DMRT test.

	SL(cm)	RL(cm)	SFW(g)	RFW(g)	SDW	RDW	SPAD
Control	30 ± 2.5 a	14.5 ± 2 b	31.15 ± 1 b	5.25 ± 0.4 b	2.74 ± 0.1 b	0.49 ± 0.01 b	51.2 ± 2 b
AGH3	38 ± 3 b	17 ± 2 a	37.67 ± 1 a	8.35 ± 0.5 a	4.54 ± 0.3 a	0.58 ± 0.01 a	55 ± 2 a
PEG Stress							
5%	23 ± 3 cd	12.1 ± 0.5 bc	21.61 ± 1 d	4.7 ± 0.1 c	2.4 ± 0.1 c	0.36 ± 0.01 d	37 ± 1 d
10%	20 ± 1.5 ed	10 ± 0.6 de	14.47 ± 0.5 f	2.07 ± 0.1 f	1.8 ± 0.1 d	0.27 ± 0.01 f	34 ± 1 ef
15%	17 ± 0.6 e	8.2 ± 0.9 e	14.26 ± 0.6 f	2.29 ± 0.3 ef	1.5 ± 0.1 e	0.25 ± 0.01 g	31 ± 1 g
PEG + AGH3							
AGH3 + 5%	25 ± 0.9 c	13.4 ± 1.4 bc	23.03 ± 0.5 c	5.8 ± 0.3 b	2.9 ± 0.1 b	0.39 ± 0.005 c	42 ± 1 c
AGH3 + 10%	23 ± 0.6 cd	11.6 ± 0.4 cd	18 ± 0.5 e	3.58 ± 0.3 d	2.2 ± 0.05 c	0.32 ± 0.005 e	36 ± 1.5 de
AGH3 + 15%	20 ± 0.9 ed	9.7 ± 0.9 de	15.6 ± 0.4 f	2.84 ± 0.1 e	1.9 ± 0.1 d	0.27 ± 0.01 f	33 ± 2 fg

Figure 3. *Cont.*

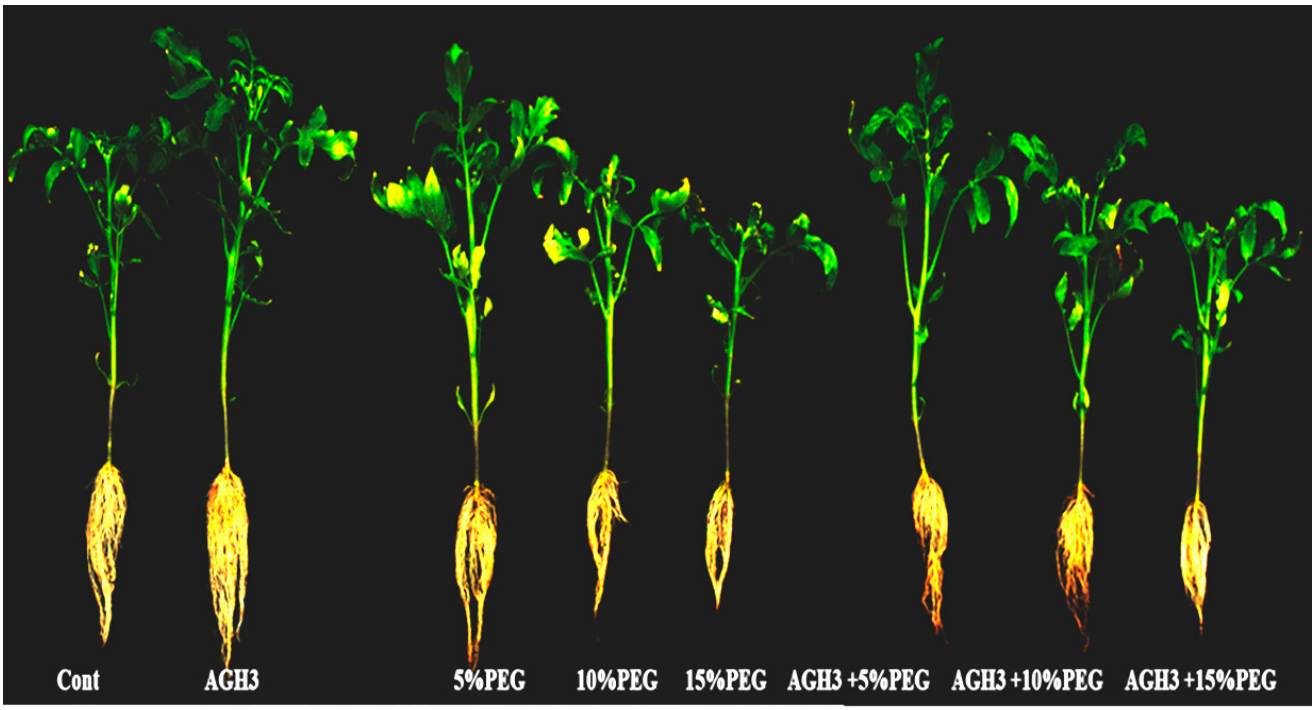

Figure 3. Effect of AGH3 on *S. lycopersicum* under normal and stressful conditions.

Similarly, under PEG stress, the AGH3-inoculated plants showed significantly (p = 0.05) higher growth attributes than the non-inoculated *S. lycopersicum* plants. The *S. lycopersicum* plants under PEG stress (5%, 10%, and 15%) showed significant decreases in SL (23.33–43.33%), RL (16.55–43.44%), SFW (30.62–54.22%), RFW (10.47–56.38%), SDW (12.40–45.25%), RDW (20.40–48.97%), and CC (27.73–39.45%). However, the *S. lycopersicum* plants inoculated with AGH3 showed significant increases in SL (8.6–17.64%), RL (10.74–18.29%), SFW (6.57–9.39%), RFW (23.40–24.01%), SDW (20.83–26.66%), RDW (7.69–8%) and CC (13.51–6.06%) as compared with the non-inoculated PEG-stressed *S. lycopersicum* plants (Table 5 and Figure 3).

3.4. Effect of AGH3 on Plant Endogenous Abscisic Acid (ABA) and Jasmonic Acid (JA)

The accumulated ABA and SA contents were assessed in *S. lycopersicum* plants exposed to normal and PEG-stressed (5%, 10%, and 15%) conditions (Figure 4). According to the results, there was no discernible variation in ABA content under normal conditions. Under different PEG concentrations, a significant increase in ABA content (2.1–4.5 fold) was observed in the non-inoculated tomato plants, while a noticeable reduction in ABA levels was recorded in the AGH3-inoculated tomato plants. Jasmonic acid showed a similar pattern to that of ABA. Under 5%, 10%, and 15% PEG stress, an increase in the JA content was observed in the non-inoculated tomato plants, whereas a decrease in JA content was noticed in AGH3-treated tomato plants (Figure 4).

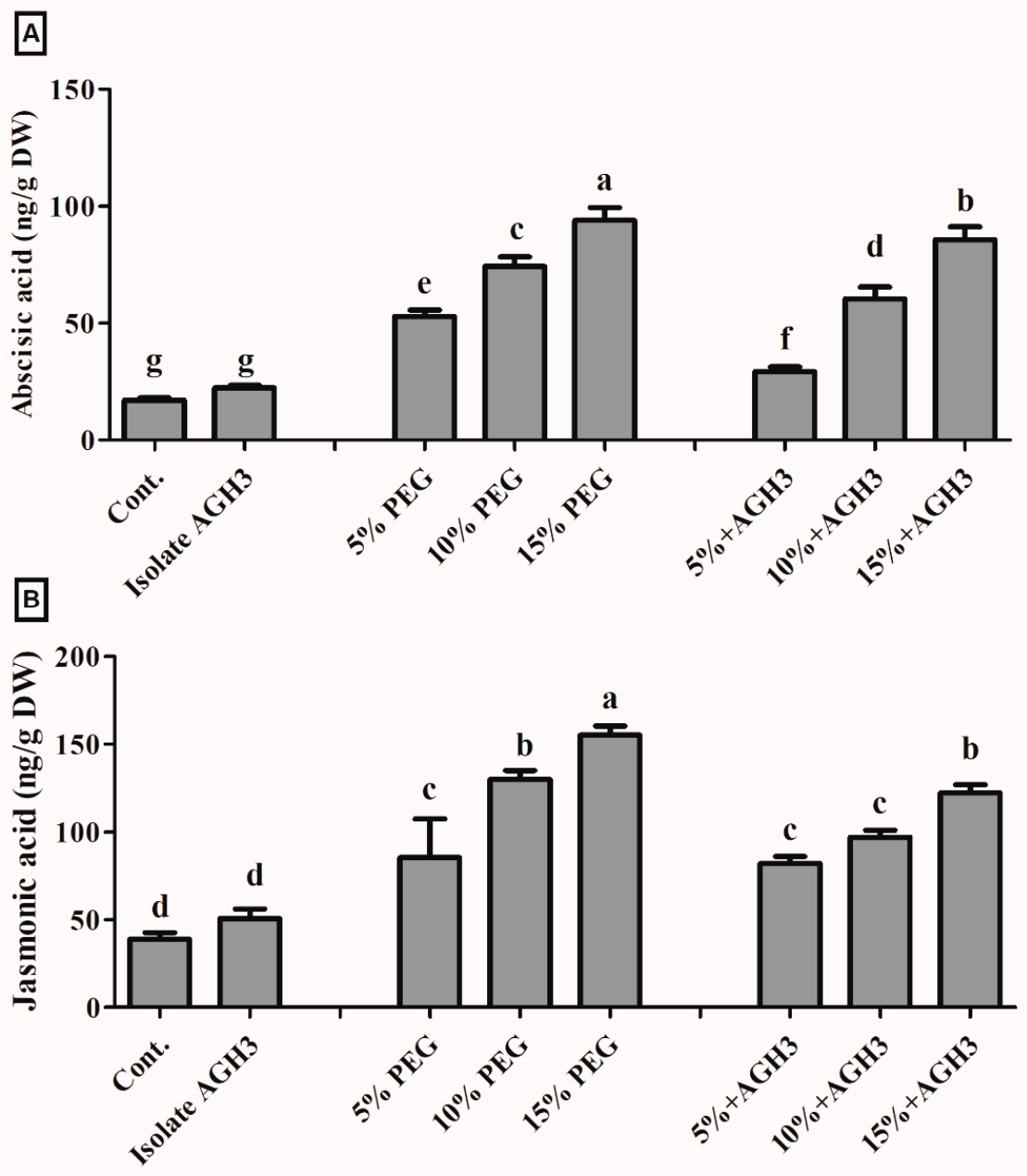

Figure 4. Effect of AGH3 on *S. lycopersicum* plant endogenous: (**A**) Abscisic acid (ABA); (**B**) jasmonic acid (JA). Different letters in the column show that values are significantly different ($p < 0.05$) from each other as evaluated from the DMRT test.

3.5. Gene Regulation under Drought Stress

Significantly ($p = 0.05$) low *SlHsfA1a* and *SlHAKT1* transcriptional levels were noticed in tomato plants under PEG treatments (Figure 5). The expressions of *SlmiR 159* (6–10-fold) and *SlHsfA1a* (1–4-fold) genes were significantly ($p = 0.05$) increased at different PEG-stressed conditions (5%, 10%, and 15%). However, higher expressions of *SlmiR 159* (1–4-fold) and *SlHsfA1a* (1–0.39-fold) were detected in AGH3-treated *S. lycopersicum* plants under PEG stress. Similarly, higher gene expression levels of *SlHAKT1* (0.26–1-fold) were observed in AGH3-associated *S. lycopersicum* plants as compared with the PEG-stressed plants (Figure 5).

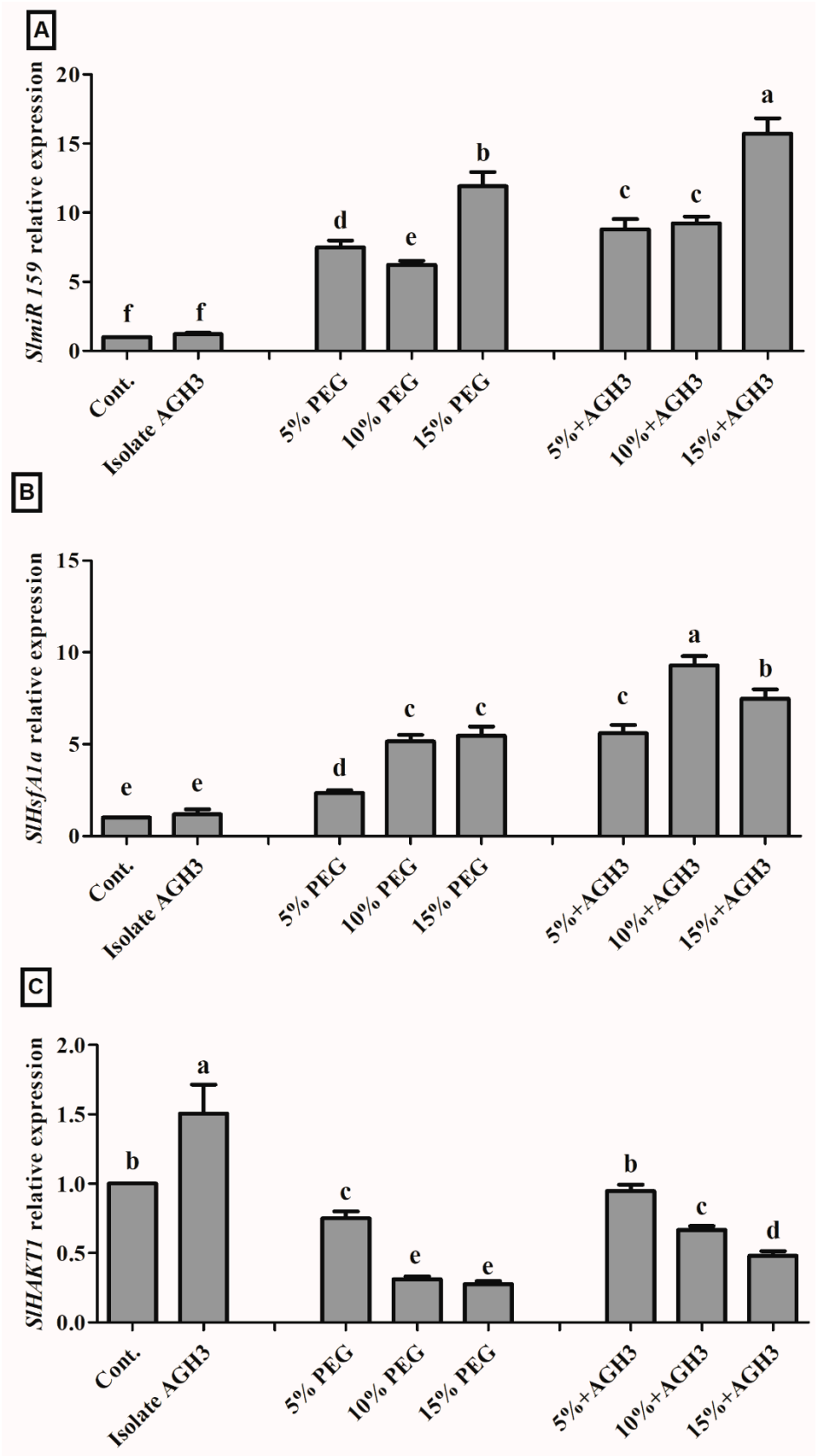

Figure 5. Gene expression of *S. lycopersicum* by using qRT-PCR under different PEG-stressed conditions with and without AGH3: (**A**) *SlmiR159*; (**B**) *SlHsfA1a*; (**C**) *SlHAKT1*. Different letters in the column show that values are significantly different ($p < 0.05$) from each other as evaluated from the DMRT test.

3.6. Molecular Identification of Multi-Trait PGP AGH3

The rhizobial strain AGH3 that promoted plant growth and development was identified at the molecular level. Based on 16S rRNA sequence identity, the rhizospheric bacterial isolate AGH3 was identified as *Microbacterium oxydans* (Supplementary File S1 and Figure S1). Furthermore, the sequence was submitted to NCBI under the accession number ON979829.

4. Discussion

Plant development and productivity are influenced by biotic and abiotic stresses [5,41]. The most significant growth constraint for agricultural crops around the world, especially in arid and semi-arid regions, is considered to be drought stress. According to the reports, by 2050, the world's temperature is predicted to increase by 4 °C. This increase in temperature will melt glaciers that, in turn, will create floods in the short term and a severe water crisis in the long run [41]. In the past, significant famines were thought to have been caused in various parts of the world by drought. In the current work, a substantial reduction in plant height and biomass accumulation were observed under drought stress conditions [53]. Moreover, it has been demonstrated that several beneficial rhizobacteria with a variety of characteristics can stimulate plant growth and provide resistance against abiotic stressors. It is interesting to note that inoculation of numerous crop plants with potent PGPR under abiotic stresses has increased over the past [54]. According to a study by Showemimo and Olarewaju [55], drought stress resulted in flower aborting, decreased fruit set, and eventually decreased crop yield. The effects of drought stress were also reported by Earl and Davis [56] in terms of reduced leaf area, stem lengthening, and root proliferation, altered plant water relations, and reduced plant growth and productivity [57]. Hamayun et al. [58] proposed that drought-induced losses in plant growth due to decreased turgor pressure could be reversed by applying multi-trait PGPR [59]. Through a variety of direct and indirect mechanisms, these beneficial bacteria grown in plant rhizosphere promote plant growth, and therefore, the role of PGPR in the management of abiotic stressors such as drought is gaining importance.

The use of AGH3, in the current study, significantly improved plant PGP attributes, including photosynthetic activity and biomass of *S. lycopersicum* under drought stress conditions. On the contrary, drought stress significantly decreased the shoot and root lengths as well as the fresh and dry weights of AGH3-free tomato plants (Table 5). Similar to this, earlier studies have demonstrated the mechanisms by which drought stress impacted the development of several crop plants [54]. As compared with plants undergoing drought stress, the selected rhizospheric strain (AGH3) considerably improved tomato plant growth, biomass, and chlorophyll contents under stress. Rhizospheric bacteria that promote plant growth have recently been employed to lower the impact of drought stress and increase crop production. PGPR rely on root exudates to fuel their metabolic activities and the diversity of PGPR is influenced by soil physiological conditions and nutrient availability in the rhizosphere [60]. These PGPR provide plants with various growth promoting benefits and act as biofertilizers and microbial plant biostimulants that are most beneficial for agricultural sustainability by promoting overall plant growth under severe drought stress.

In the past, various scientists have observed the ability of PGPR to produce IAA, solubilize phosphorus, and produce siderophores [61]. The bacteria that were applied in this experiment played an active role in the formation of siderophore, IAA, GA, and solubilization of phosphate. Phosphate solubilization, IAA, GA, and siderophore production capabilities in PGPR alleviate drought stress-associated damage in host plants by providing them with sufficient amounts of soluble phosphate, iron, and hormones [62]. The microbial inoculants that are administered to host roots should contain enough growth regulators to have an impact on subsequent plant growth and development. The plant may have simple access to additional phytoregulators and additional bioactive secondary metabolites that it can absorb and use to promote root development, cell maturation and differentiation, tissue maturation and differentiation, and subsequently, host growth. Additionally, our bac-

terial isolate produced IAA, which significantly decreased the negative impacts of drought stress on tomato plants. Numerous earlier studies have revealed the positive effects of gibberellins on host growth in unfavorable environmental conditions. Although there are various types of GA, only GA1, GA3, and GA4 are thought to be biologically active forms. Through a reduction in stress hormones (such as ABA), plant development can be encouraged. Plants become more sensitive to stomatal conductance as a result of controlling stress hormones by releasing chemical signals as soon as they suspect stress (Figure 4). A similar study by Uzma et al. [62] reported that the application of PGPR (*P. aeruginosa*) could facilitate regulating the level of phytohormones by synthesizing and secreting them to improve the root system architecture of plants and to assist them to thrive during drought stress condition.

For the development of sustainable agricultural methods, the identification of drought tolerance genes is critical [63,64]. It has been shown that it is possible to genetically modify a variety of crop plants for agricultural use in drought-prone environments [65]. The regulation of transcriptional re-programming associated with plant stress responses is significantly influenced by some of the gene families [66]. In addition, a genome-wide transcriptome investigation of the tomato plant found that, during drought stress, between the roots and the shoots, different hormone-related genes were expressed. In plants, different genes increase tolerance and protect protein function when exposed to stressful conditions. Some genes are crucial for preserving and re-establishing biologically important macromolecules and steadying the state of stressed plants. Numerous genes are activated in response to abiotic stress conditions in various crop plants, which has been found to result in PGPR-mediated stress resistance. In crop plants treated with PGPR, researchers have found that abiotic stress increased the level of heat shock transcription factors (HSFs) and decreased the level of heat shock protein (HSP) transcripts. Similarly, *SlHsfA1a* was upregulated in tomato plants under stressful conditions as compare with untreated control plants. In our study, gene expression was analyzed by using qRT-PCR of *S. lycopersicum* under different PEG-stressed conditions with and without AGH3 inoculation. Candidate genes, such as *SlmiR* 159 and *SlHsfA1a* revealed higher expressions of *SlmiR 159* and *SlHsfA1a* under drought stress (Figure 5). However, higher expressions of *SlmiR 159* and *SlHsfA1a* were detected in AGH3-treated *S. lycopersicum* relative to solely PEG-stressed plants. Similarly, when tomato plants were exposed to PEG stress, *SlHAKT1* gene expression showed that *SlHAKT1* was downregulated as compared with control plants, while higher gene expression of *SlHAKT1* was observed in tomato plants inoculated with AGH3 as compared with solely PEG-stressed plants.

5. Conclusions

The results of the present research should be very useful to the agriculture sector, due to the potential of utilizing multi-trait rhizobacterium (AGH3) as a microbial plant biostimulant that can significantly improve stress tolerance and enhance growth and development under drought stress conditions. The PGPR strain AGH3 that promoted plant growth and development was identified as *Microbacterium oxydans*, and its active roles in the formation of siderophore, IAA, GA, and solubilization of phosphate were revealed. The strain AGH3 demonstrated the possible ability to mitigate drought stress-associated damage in *Solanum lycopersicum* and to improve plant growth attributes at the physiological, biochemical, and molecular levels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123224/s1. Table S1: List of primers used in the present study; Supplementary file S1: Sequence of bacterial isolate AGH3; Figure S1: Phylogenetic analysis of multi-trait PGP AGH3. The homology of nucleotide sequences was compared using the BLAST search software. The 16S rRNA region were employed in the current investigation were obtained from NCBI and used to build the tree using the MEGA 6.0 software. The nodes in the phylogenetic tree were supported statistically by the bootstrap replications (1K).

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