Plant Soil (2020) 452:423–440
https://doi.org/10.1007/s11104-020-04582-5

REGULAR ARTICLE

Drought-tolerant plant growth-promoting rhizobacteria isolated from jujube (*Ziziphus jujuba*) and their potential to enhance drought tolerance

Min Zhang · Lin Yang · Ruqian Hao · Xiaoxiong Bai · Ying Wang · Xuan Yu

Received: 28 May 2019 / Accepted: 24 May 2020 / Published online: 6June2020

Abstract

Background and aims Plant growth-promoting rhizobacteria (PGPR) have important roles in improving plant growth and alleviating stress induced damage under drought stress conditions. The aims of this study are: 1) to isolate and identify drought-tolerant PGPR from rhizosphere soil of jujube, a drought-tolerant plant grown in semi-arid and arid regions; and 2) to evaluate the effects of inoculation with the isolated strains on the growth and physiological responses of jujube seedlings under drought stress conditions.

Methods Rhizosphere bacteria with 1-amino-cyclopropane-1-carboxylate (ACC) deaminase activity were isolated from the rhizosphere of jujube and were identified by 16S rDNA sequencing analysis. Then, the isolates were screened for drought tolerance and plant growth promoting activities. The growth and physiological changes of jujube under drought stress, including the plant height, shoot and root dry matter, malondialdehyde (MDA), indoleacetic acid (IAA), abscisic acid (ABA) content, superoxide dismutase (SOD), and peroxidase (POD) activity, were also detected.

Results Eight ACC deaminase-producing bacterial strains were isolated and identified as *Pseudomonas*, *Bacillus*, and *Serratia*. Under drought stress conditions, *Pseudomonas lini* and *Serratia plymuthica* significantly increased plant height, shoot and root dry matter, and relative water content. Moreover, malondialdehyde and ABA levels were decreased, and antioxidant enzyme activities were increased. In addition, they also enhanced soil aggregate stability. The best effect was observed with mixed inoculation.

Conclusions Strains of *Pseudomonas lini* and *Serratia plymuthica* played an important role in enhancing tolerance of jujube seedling and can be considered as promising bioinoculants of jujube.

Keywords Plant growth-promoting rhizobacteria (PGPR) · 1-aminocyclopropane-1-carboxylate (ACC) deaminase · Drought stress · *Ziziphus jujuba* · *Pseudonomas* sp. · *Serratia* sp.

Responsible Editor: Anna Maria Pirttila.

M. Zhang · L. Yang · R. Hao · X. Bai · Y. Wang · X. Yu
Department of Forestry, College of Forestry, Northwest A&F University, Yangling, China

M. Zhang · L. Yang · R. Hao · X. Bai · Y. Wang · X. Yu
Key Comprehensive Laboratory of Forestry, Yangling, Shaanxi Province, China

M. Zhang · L. Yang · R. Hao · X. Bai · Y. Wang · X. Yu (✉)
Key Lab Silviculture Loess Plateau State Forestry, Yangling, Shaanxi, China
e-mail: yuxnwsuaf@163.com

Introduction

With the change in global climate, drought stress is one of the major limiting factors on plant growth and yield in agricultural production worldwide, thus affecting the world's food security (Vurukonda et al. 2016; Chandra et al. 2018a). At present, there are many strategies to

cope with drought, including water-saving irrigation, breeding for drought-tolerant varieties, and regulating crop calendars. However, these strategies are costly, time consuming, labor intensive and difficult to use in practice.

The application of plant growth-promoting rhizobacteria (PGPR) is one of the efficient ways to increase drought tolerance of plant. Many studies have reported that inoculation with PGPR is useful to help enhance drought tolerance of plants (Garcia et al. 2017; Khan et al. 2018a, b). It was reported that water deficit could induce ethylene production in plant (Ravanbakhsh et al. 2018). The increase of ethylene content would restrain root, shoot development and leaf expansion (Li et al. 2017), which consequently impact plant growth. 1-aminocyclopropane-1-carboxylic acid (ACC) is a precursor for ethylene synthesis (Vanderstraeten and Van Der Straeten 2017). The ACC deaminase enzyme produced by PGPR plays an important role in reducing ACC content through degrading ACC into α-ketobutyrate and ammonia (Danish and Zafar-ul-Hye 2019) and then reduces ethylene production in plants. Therefore, isolation of PGPR producing ACC deaminase activities is of great importance in helping plants to alleviate the effects of stress-generated ethylene. In addition, these beneficial microorganisms could induce changes in morphology, physiology, and biochemistry in plants in other ways: (1) by producing exopolysaccharides (EPSs) and phytohormones and (2) by inducing the accumulation of osmolytes, antioxidants, and alteration of root morphology (Vurukonda et al. 2016) and eventually alleviating drought stress. However, the effectiveness of inoculated PGPR mainly depends on colonization of the rhizosphere, especially under stress conditions (Yuan et al. 2018; Batista et al. 2018). Most microorganisms might not be actively growing in a water deficit environment (Williams and Rice 2007; Wang et al. 2008). Therefore, drought-tolerant PGPR producing ACC deaminase might be well adapted to the stressful environment and function well. In addition, studies on the inoculation effect of PGPR on alleviating drought stress have chiefly focused on crops, such as sugarcane (Chandra et al. 2018b), maize (Skz et al. 2018), soybean (Martins et al. 2018), foxtail millet (Niu et al. 2018), and chickpea (Khan et al. 2018c). The impacts of PGPR on plant growth and the alleviation of drought stress in jujube have not been evaluated. Additionally, few studies have comprehensively revealed the interrelationships between PGPR and plants under stress conditions. To the best of our knowledge, this is the first report to detect the effect and mechanism of PGPR on jujube seedlings under drought stress.

Jujube (*Ziziphus jujuba*) is an important perennial fruit tree that is widely cultivated in arid and semiarid regions, such as the Loess Plateau, which lies in semi-arid Northwest China. This is mainly because (1) the fruits are rich in sugar, flavonoids, minerals (e.g. calcium, iron). (2) The species plays an important role in soil and water conservation. (3) The tree species is cold- and drought-tolerant, and well adapted to the area. Although jujube is a drought-tolerant plant, drought stress becomes more of a threat in arid regions and results in limited jujube growth and reduced productivity. Pruning (to reduce canopy leaf area) (Chen et al. 2016), gene breeding (Ji et al. 2019), and plant cover (Pan et al. 2017) are often used as methods of alleviating drought stress in jujube. However, these methods are time-and labor-consuming. It was reported that rhizosphere microbial populations distributed in drought-tolerant plants helped their host plants adapt to drought stress conditions (Huang et al. 2017; Misra et al. 2019). According to previous studies, we assumed that bacteria living in the rhizosphere of jujube grown in arid areas may have adapted to a drought environment due to long term water shortage. Thus, they may play a vital role in enhancing tolerance to drought stress in jujube.

Therefore, the main aim of the present study was to (1) isolate and identify drought-tolerant bacterial strains from the jujube rhizosphere in arid land in the Loess Plateau, Northwest China and (2) evaluate the effect of the strains on the drought response of jujube seedlings under simulated drought stress conditions.

Materials and methods

Isolation of rhizobacteria with ACC deaminase activity

The bacterial strains were isolated from the rhizosphere soil of 11-year jujube trees (*Ziziphus jujuba*), which were growing in arid land in the Gully Region of the Loess Plateau. The sampling area is characterized by a temperate continental monsoon climate. The annual rainfall is 450 mm and mainly occurs from July to September. The soil is classified as loess soil according to the USDA soil taxonomy (US Department of Agriculture 2010). The basic characteristics of the

sampling sites were as follows: pH = 8.35, total organic carbon = 4.18 g·kg^{-1}, total nitrogen = 0.31 g·kg^{-1}, ammonium nitrogen = 2.85 mg·kg^{-1}, and available phosphorus = 1.15 mg·kg^{-1}. For rhizosphere soil samples, the bulk soil was removed by gently shaking the roots. A soil suspension was obtained by dipping five grams of roots into flasks containing 100 mL sterilized distilled water followed by shaking at 120 rpm for 30 min. The suspension was serially diluted to the concentration of 10^{-6}•mL^{-1}. 0.1 mL suspension of each dilution was spread on Nutrient agar medium containing 0.5% peptone and 0.3% beef extract and incubated at 28 °C for 48 h. The bacterial colonies with different morphological features were isolated and purified. Isolates were kept in Nutrient Agar slants at 4 °C and were used for further study.

Generally, selection of ACC deaminase producing bacteria mainly depended on the ability to utilize ACC as the sole nitrogen source (Glick et al. 1995). In order to screen the capacity of using ACC as sole nitrogen source, the method of Cedeno-Garcia et al. (2018) was carried out. Bacterial strains were inoculated onto three agar mediums: (1) DF agar medium supplemented with 3 mM ACC as sole nitrogen source; (2) DF agar medium supplemented with $(NH_4)_2SO_4$ as sole nitrogen source, and (3) DF agar medium without nitrogen source, respectively. The positive control was DF agar medium supplemented with $(NH_4)_2SO_4$. The negative control was DF agar medium without nitrogen source. Five microlitres of bacterial suspensions were placed on each medium and cultured at 28 °C for 3 days. The growth on the plate was checked daily. The bacterial strains that can grow in DF medium were considered as the potential of containing ACC deaminase activity (Qin et al. 2014; Huang et al. 2017).

Based on the above experiment, the ACC deaminase activities in eight isolates were detected quantitatively. The ACC deaminase activities of the bacterial strains were evaluated by detecting the amounts of α-ketobutyrate, which were generated by the degradation of ACC. A detailed protocol was used as described by Penrose and Glick (2003). Isolates were grown in 5 mL of Tryptic soybean broth (TSB) medium at 28 °C until they reached the stationary phase. Then, the cells were collected by centrifugation, washed twice with 0.1 M Tris-HCl (pH = 7.5), suspended in 2 mL of modified DF minimal medium with 3 mM ACC as the sole source of nitrogen and incubated at 28 °C with shaking for another 24 h. ACC deaminase enzymatic activity was determined by measuring the production of α-ketobutyrate generated by the degradation of ACC. The protein content in toluenized cells was detected by Bradford reagent (Bradford 1976). Bovine serum albumin was used for the standard curve. The ACC deaminase activities were expressed as μmol of α-KB mg^{-1} protein h^{-1}.

Bacterial identification using 16S rDNA sequences

Characterization of the eight ACC deaminase-producing strains to the genus level was performed by partial sequencing of the 16S ribosomal DNA gene, which was amplified by PCR using the universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5'-GGTTACCTTGTTACGACTT-3′) (Islam et al. 2014; Niu et al. 2018). PCRs were carried out in a total volume of 25 μL reaction mixture containing 1× reaction buffer, 10 pM of each primer, 2.5 mM dNTPs, 1 U of Taq DNA polymerase (Tiangen Biotechnology Ltd., Beijing, China), and 25 ng of template DNA. Amplification was carried out in a thermocycler (BioRad, USA) programmed as follows: initial denaturation at 94 °C for 4.5 min, followed by 35 cycles included denaturation at 94 °C for 1 min, annealing at 57 °C for 30 s, and extension at 72 °C for 2 min. A final extension step was carried out at 72 °C for 7 min. Purified PCR products were sequenced using an ABI3730-XL DNA sequencer from Sangon Biotechnology Ltd. (Shanghai, China). The obtained 16S rDNA sequences were aligned and compared with the known sequences in the NCBI nucleotide database by the BLAST algorithm to find closely related bacteria. Identification at the species level was considered as a detected 16S rDNA sequence similarity score ≧99% with strain sequences deposited in GenBank. A phylogenetic tree was established through the neighbor-joining method from distance matrices.

Assessment of drought tolerance and plant growth promoting traits

Nutrient broth medium (NB) with different water potentials (0, −0.05, −0.30, −0.49, and − 0.73 MPa) was prepared by adding different concentrations (0, 10, 15, 20, 25% (*w*/*v*)) of PEG 6000 (Michel and Kaufmann 1973) and then inoculated with 1% overnight cultivated bacterial cultures. These cultures were incubated at 28 °C under shaking conditions (120 rpm) for 24 h. The growth

of bacterial isolates was determined by measuring the optical density at 600 nm using a spectrophotometer. To detect phosphate solubilization, after activation for 24 h, the bacterial strains were spotted on Pikovskaya's agar media including 2% tricalcium phosphate and were observed for the appearance of a clear halo zone around their colonies after 72 h of incubation at 28 °C (Sandhya et al. 2009). For quantitative measurement, 1 mL of bacterial culture (1×10^8 cfu·mL^{-1}) was inoculated in a 300 mL flask with 80 mL of Pikovskaya's liquid medium. Flasks were incubated on a gyratory shaker at 200 rpm for 3 days at 28 °C. The cellular P and phosphorus in the supernatant were measured by the Mo-blue colorimetric method (Watanabe and Olsen 1965). Potential for nitrogen fixation was evaluated preliminarily using nitrogen-free medium (Day and Döbereiner 1976) by the strain growth test in this medium. It has been reported that bacterial strains that can grow in nitrogen free medium are considered presumptive for N_2 fixation (Cattelan et al. 1999). The bacterial were grown in this medium at 28 °C for 3 days. The bacterial strains that could grow in this medium after incubating 3 days were considered to have potential capacity for nitrogen fixation (Cattelan et al. 1999). IAA production was examined by the colorimetric method, using Salkowsky coloring reagent (Malik and Sindhu 2011). To quantitatively determine EPS production, overnight bacterial cultures were inoculated in 50 mL of TSB and incubated on a shaker at 200 rpm for 3 days at 28 °C. Extraction of the released and intracellular EPS mainly based on the procedure described by Ali et al. (2014). The exopolysaccharide was quantified according to the phenol-sulfuric acid method (Dubois et al. 1956).

Pot experiment

According to the results, the two best-performing bacterial strains *Pseudomonas lini* DT6 (accession number: CGMCC 1.18402) and *Serratia plymuthica* DT8 (accession number: CGMCC 1.17845) which could efficiently colonize in the jujube rhizosphere were selected for pot experiments. Inocula were prepared by incubating the bacterial strains in NB to the mid-exponential growth phase followed by diluting the cultures with sterile distilled water to a final concentration of 10^8 cfu mL^{-1}. A sandy loam was collected from the jujube experimental station of Northwest A&F University in arid land in Qingjian County, Shaanxi Province (37°6′36"N, 110°4′48″E) and used as the potting soil. The soils were sterilized by autoclaving at 121 °C for 4 h (Zhang et al. 2016). The purpose of using sterilized soil was to avoid the interference of the native microbiome in the rhizosphere and improved colonization efficiency of the bacteria inocula. The chemical properties of the soil were as follows: pH 7.82, organic matter 11.09 g·kg^{-1}, total nitrogen 0.87 g·kg^{-1}, total phosphorus 0.35 g·kg^{-1}, available P 4.56 mg·kg^{-1}. Urea and potassium nitrate were mixed with the potted soil based on the doses of N (300 mg·kg^{-1} soil) and potassium (200 mg·kg^{-1} soil, calculated as K_2O).

A pot experiment was carried out with a two-factor completely randomized design with 5 replications. For drought treatment, 60 plants were randomly divided into three groups (20 plants in each group). Plants in the three groups were grown at 80% (control, ND), 60% (moderate drought stress, MD) or 40% (severe drought stress, SD) field capacity, respectively. According to the method of Jia et al. (2015), each pot (26.0 cm diameter × 36.5 cm height) was weighed daily, and the amount of water equal to the losses of transpiration and soil evaporation was added to achieve the target field capacity. For bacterial inoculants, there were four treatments as follows: (1) control without bacteria (CK); (2) *Pseudomonas lini* (T1); (3) *Serratia plymuthica* (T2); and (4) a 1:1 volume mixture of T1 and T2 (T3). Under all of the drought treatments, 20 plants were divided into four groups (5 plants each group), and the plants in each group were inoculated with one of four bacterial inoculants. A 100 mL bacterial suspension was inoculated in to the middle of seedling roots using a syringing method (Aslantas et al. 2007). The same volume of sterilized nutrient broth was applied for the control plants. The drought treatment lasted for 20 days. Subsequently, jujube seedlings were harvested. Growth promoting effects were evaluated by measuring plant height, shoot and root dry weight, nitrogen and phosphorus content. Seedlings from the same treatment were also used for assessment of the physiological indexes, including malondialdehyde and antioxidant activities. In addition, the ratio of root adhering soil (RAS) to root tissue, soil aggregate stability, and relative water content of jujube seedlings under serious drought stress conditions were determined after the stress experiment was over.

Parameters measured

Soil chemical properties were detected by the method of Cui et al. (2018). Soil pH was measured in a 1:2.5 soil:

water suspension after 20 min of oscillation by a pH meter (Metrohm 702, Herisau, Switzerland). For the measurement of soil organic matter, 0.5 g of air-dried soil was digested in mixture containing 5 mL 0.8 M $K_2Cr_2O_7$ and 5 mL H_2SO_4 at 170–180 °C for 5 min and was then titrated with 0.2 M $FeSO_4$. Total nitrogen was detected by the Kjeldahl method. Total phosphorus content was estimated by molybdenum blue colorimetry after digestion with a mixture of 2 mL $HClO_4$ and 3 mL H_2SO_4. For determination of available phosphorus, 2.5 g of air-dried soil was supplemented with 50 mL 0.5 M sodium bicarbonate and one spoon of active carbon without phosphorus and was vibrated at 25 °C for 30 min. Then the filtrate of the solution was measured using molybdenum blue colorimetry.

Shoot and root dry weight was analyzed after drying in a forced hot-air oven at 70 °C for two days (Calvo-Polanco et al. 2016).

The contents of nitrogen and phosphorus in the leaves were analyzed according to the protocol of Chandra et al. (2019). Oven-dried leaf samples were ground through a 1 mm sieve. Total nitrogen content was detected using the Kjeldahl method. The phosphorus content was determined using molybdenum blue colorimetry after digestion with nitric and perchloric acids.

The MDA level in the leaves was detected as described by Li et al. (2017). One gram of fresh leaves was extracted and homogenized with 20 mL of 0.1% trichloroacetic acid (TCA) solution and centrifuged for 10 min at 12,000 g. Four milliliters of TCA solution including 5% thiobarbituric acid (TBA) was mixed with 1 mL of supernatant. The mixture was heated at 95 °C for 30 min and then cooled to room temperature. The absorbance of the mixture was recorded at 532 nm and 600 nm. The MDA content was calculated based on an extinction coefficient of 155 mM^{-1} cm^{-1}.

Antioxidant enzymes, superoxide dismutase (SOD) and peroxidase (POD) enzymes of leaves were extracted according to Abedi and Pakniyat (2010). Fresh leaves (0.5 g) were homogenized with pre-cooled Tris-HCl buffer (pH 7.5) containing 5% sucrose and 0.1% mercaptoethanol. The homogenate was centrifuged at 12,000 g for 20 min at 4 °C. The supernatants were used for determination of SOD and POD activities. The SOD activity was measured at 560 nm via nitro blue tetrazolium chloride (NBT) photoreduction (Beyer and Fridovich 1987). The POD activity was determined at 470 nm by guaiacol oxidation caused by H_2O_2 (Zhou and Leul 1998).

Leaf phytohormones (indole-3-acetic acid (IAA)) and abscisic acid (ABA) were quantified with the Phytodetek-IAA and Phytodetek-ABA Immunoassay kits (Agdia, Elkhart, IN), respectively. Phytohormone contents were analyzed according to the manufacturer's protocol.

The RAS/RT ratio was calculated based on the method of Sandhya et al. (2009). The RASs were separated by washing roots in distilled water. The root dry weights were recorded after drying at 105 °C. Soil water-stable aggregates were fractionated using the wet sieving method. The amounts of water-stable aggregates (>0.25 mm) were calculated by subtracting the coarse sand and root fragments remaining on the sieve. Soil aggregates were oven-dried and transferred to dispersion cups and stirred for 10 min with 10% sodium hexametaphosphate. Aggregate stability was evaluated based on the amounts of water-stable aggregates (Bartoli et al. 1991; Sandhya et al. 2009). The relative leaf water content was determined as the method of Wang et al. (2016), the fresh leaves in vitro were firstly weighed and then placed in distilled water for 12 h and turgid weight were obtained. The leaves were dried at 80 °C to constant weight and dry weight were detected. The relative leaf water content was calculated according to Sharp et al. (1990).

Isolation of the *acd*S mutant strain and determination of stress-induced ethylene

The wild strains DT6 and DT8 were used for isolating their corresponding *acd*S mutant strains according to the methods of Sarkar et al. (2018a, b). Bacterial cells harvested from overnight culture were washed twice using the sterile Tris-maleate (TM) buffer (pH 6.0) and then were suspended again. Then, 4 mL bacterial suspension (diluted to 10^8 cfu mL^{-1}) added 1 mL 100 μg·mL^{-1} N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) solution was incubated statically at 28 °C for 24 h. After incubation, the bacterial cells were suspended with 20 mL DM medium supplemented with ACC as the sole nitrogen source with 400 μg·mL^{-1} amoxicillin and incubated at 30 ± 2 °C with shaking for 24 h. After incubation, the colony was isolated using DM medium having $(NH_4)_2SO_4$ as nitrogen source. The *acd*S mutants were selected through the replica-plating method using DM medium with 3 mM ACC as sole nitrogen source and DF medium supplemented with $(NH_4)_2SO_4$ as nitrogen source. The mutants DT6s and DT8s could not grow in minimal medium due to

missing the ability of utilizing ACC, but able to grow in complete medium with $(NH_4)_2SO_4$ as nitrogen source.

In order to evaluate the effects of strains and their *acd*S mutants on the reduction of stress ethylene of jujube seedlings under drought stress conditions, a pot experiment was conducted. The experiment design, bacterial inoculum preparation and inoculation method of the *acd*S mutants were the same as described above. The ethylene content in the leaf was detected according to the methods of Grodzinski et al. (1982) and Gupta and Pandey (2019). 3 g of jujube seedling leaves per treatment were put into sealed tubes and incubated at 25 °C for 3 h. From each tube, 1 mL of gas sample was collected with a syringe and injected into the gas chromatographic analyzer. The rate of ethylene production was presented as nmol g^{-1} fresh weight (FW) h^{-1}.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using the SPSS package (version 11.0). Means were compared using Duncan's multiple-range tests at a significant level of $P < 0.05$.

Results

Isolation and identification of ACC deaminase-producing bacteria

A total of 86 bacterial strains were isolated from the rhizosphere soil of jujube. Among them, there were 8 ACC-deaminase-producing bacterial strains. As shown in Table 1, these isolates showed different ACC deaminase abilities. The highest ACC deaminase activity was exhibited by isolate DT6 (234.98 ± 0.59 (μmol α-KA/(mg Pr·h)), followed by DT8 (171.39 ± 0.45 (μmol α-KA/(mg Pr·h)), and then DT2 (129.06 ± 0.36 (μmol α-KA/(mg Pr·h)). Then, the 8 ACC deaminase-producing strains were identified by sequencing of the 16S rDNA gene. Based on the sequence of the 16S rDNA gene, these strains revealed a high similarity to known bacteria belonging to the genera *Pseudomonas* (6 isolates), *Bacillus* (1 isolate), and *Serratia* (1 isolate). *Pseudomonas* strains were particularly dominant (Table 2, Fig. 1).

Characterization of drought tolerance, other plant growth promoting properties, and EPS production

The growth of these isolates was affected by drought stress. However, three isolates DT3, DT6 and DT8 were able to grow at −0.30 MPa water potential (Table 1). Compared to non-stress conditions, the ACC deaminase activities of the three strains were significantly reduced by 30.42%–55.84% at −0.30 MPa water potential (data not shown). In addition, all 8 strains were grown in nitrogen-free medium and showed potential for nitrogen fixing abilities. The strains DT6 and DT8 showed higher IAA secretion (30.82 ± 0.34 and 45.75 ± 0.50 μg·mL^{-1}) and phosphate solubilization abilities (69.16 ± 1.11 μg·mL^{-1} and 60.10 ± 1.56 μg·mL^{-1}), but they also produced exopolysaccharides (Table 1). Thus, DT6 and DT8 were selected for further experiments.

Table 1 ACC deaminase activity and plant growth promoting characteristics of the isolates

Strain	ACC deaminase (μmol α-KA/(mg Pr•h))	Drought tolerance (OD_{600} at −0.30 MPa)	IAA synthesis (μg•mL^{-1})	Nitrogen fixation	Soluble phosphorus circle(mm)	Phosphate-dissolving (μg•mL^{-1})	EPS production (mg•mg^{-1}protein)
DT1	46.78 ± 0.46f	–	33.90 ± 0.35b	+	12.80 ± 0.36 cd	67.43 ± 1.12b	3.92 ± 0.10
DT2	129.06 ± 0.36c	–	13.52 ± 1.02e	+	19.93 ± 0.57a	79.50 ± 1.05a	4.34 ± 0.22
DT3	34.55 ± 0.88 g	0.82	25.11 ± 0.79d	+	12.00 ± 0.46de	60.41 ± 0.90 cd	–
DT4	67.49 ± 0.39e	–	15.07 ± 0.33e	+	12.10 ± 0.40de	70.19 ± 1.41b	6.39 ± 0.18
DT5	95.95 ± 0.58d	–	8.80 ± 0.13f	+	11.17 ± 0.35e	63.42 ± 1.04c	–
DT6	234.98 ± 0.59a	1.45	30.82 ± 0.34c	+	14.20 ± 0.30b	69.16 ± 1.11b	10.25 ± 0.23
DT7	14.23 ± 0.35 h	–	24.68 ± 0.50d	+	12.77 ± 0.74 cd	52.75 ± 0.83e	–
DT8	171.39 ± 0.45b	1.21	45.75 ± 0.50a	+	13.83 ± 0.35bc	60.10 ± 1.56d	7.68 ± 0.16

Values are presented as the mean ± standard deviation ($n = 3$). Different lowercase letters indicate significant differences according to Ducan′ range test at $P < 0.05$ level

Table 2 The 16S rDNA sequence similarities of the isolated strains

Bacteria NO.	NCBI closest match (accession No.)	Accession number	Sequence identity %
DT1	*Pseudomonas fluorescens* (KJ027533)	MF631836	99%
DT2	*Pseudomonas koreensis* (KC790275)	MF631838	99%
DT3	*Pseudomonas putida* (KU977141)	MF631844	99%
DT4	*Pseudomonas fluorescens* (KJ027533)	MF631840	99%
DT5	*Pseudomonas putida* (KU977141)	MF631841	99%
DT6	*Pseudomonas lini* (KM349410)	MF631842	99%
DT7	*Bacillus filamentosus* (KF265351)	MF631843	99%
DT8	*Serratia plymuthica* (AJ233433)	MF631835	99%

Growth response of jujube seedlings

The effects of inoculation with PGPR on plant growth are shown in Table 3. The plant height, shoot and root dry matter, and nitrogen and phosphorus contents significantly decreased with the increase in drought stress intensity. However, inoculation with bacterial inoculants significantly enhanced the plant height, shoot and root dry matter, nitrogen, and phosphorus content compared with the control. Particularly under 40% field capacity (severe drought stress), these indexes increased by 49.68%–82.95%, 53.52%–86.62%, 27.31%–49.45%, and 12.52%–26.97%, respectively. The maximum index values were observed in plants co-inoculated with *Pseudomonas lini* and *Serratia plymuthica* (T3) for all water treatments. In addition, there were no significant differences between inoculated and non-inoculated plants under sufficient water conditions. With the enhancement of drought stress intensity, the root/shoot ratio significantly decreased. Under severe drought stress conditions, the root/shoot ratio decreased by 9.66%–17.24%.

Antioxidant enzyme activity and MDA contents

As shown in Table 4, the SOD and POD activities showed the same trend. When the water capacity was 80%, the differences were small between bacterial inoculated and untreated seedlings. However, bacterial inoculation significantly increased the enzyme activities under drought stress conditions compared to noninoculated seedlings for all bacterial treatments, indicating the ability of PGPR strains to alleviate drought stress. Among the PGPR strains, mixed inoculation had the greatest effect, with increases in SOD and POD activities of 5.50%–22.55% and 16.67%–37.50% compared with noninoculated seedlings, respectively.

Under no water stress conditions, the MDA content remained at a low level in all treatments. When the plants were subjected to water stress, the MDA content increased gradually. Inoculation with the three bacterial strains had important effects on retraining the content of MDA under drought stress conditions. The MDA content was significantly lower than that of the control under moderate and serious drought stress conditions. The minimum MDA content occurred with the combined microbial inoculation treatment.

IAA and ABA contents

The effects of inoculation with PGPR strains on IAA and ABA contents are presented in Table 5. IAA content showed a decreasing trend for treatments with bacteria. However, the IAA contents of inoculated seedlings were significantly higher than those of noninoculated seedlings under drought stress conditions. Especially under severe stress conditions, the IAA content of the inoculated treatment increased by 56.37%–87.62% compared with the control.

Drought stress conditions increased the content of ABA in seedlings. Inoculation with the three bacterial inoculants decreased the synthesis of ABA in jujube seedlings. The ABA contents were markedly lower by 9.28%–22.01% than noninoculated seedlings under drought stress conditions. The mixed inoculation was more effective at inhibiting the synthesis of ABA than the single inoculations.

RAS/RS ratio, soil aggregate stability, and relative water content

Inoculation of T1, T2, and T3 had positive effects on the RAS/RT ratio, soil aggregate stability, and relative water content of jujube seedlings, and the more arid the soil, the stronger the positive effects. Under serious drought stress conditions, the RAS/RT ratio,

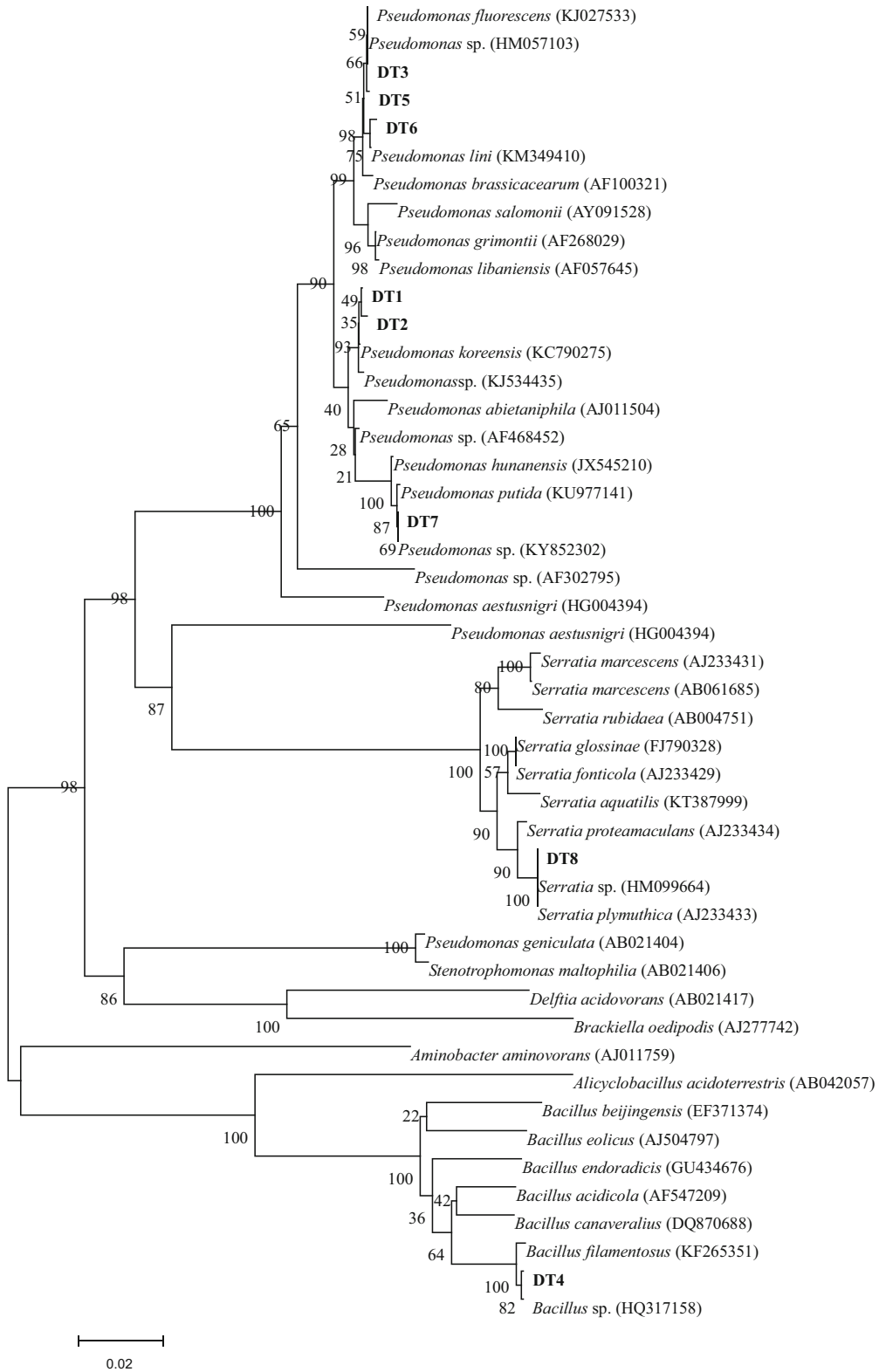

Fig. 1 Phylogenetic analysis of the eight ACC deaminase producing strains isolated from the rhizosphere of jujube

Table 3 Effects of the two drought-tolerant PGPR on growth parameters and nutrient contents of jujube seedlings under different drought stress

Drought stress	Treatment	Plant height (cm)	Shoot dry matter weight (g)	Root dry matter weight (g)	Root/Shoot ratio	TN[a] (mg·g^{-1} DW)	TP (mg·g^{-1} DW)
ND[b]	CK[c]	14.10 + 1.05g[d]	35.41 + 0.94de	44.13 + 1.29e	1.25 + 0.0058bcd	21.06 + 1.66bcd	8.34 + 0.16 cd
	T1	20.65 + 0.78ab	40.52 + 0.84b	50.49 + 0.68bc	1.25 + 0.0058bcd	27.33 + 0.58a	9.27 + 0.22ab
	T2	18.51 + 0.96 cd	39.34 + 1.11bc	48.48 + 0.93 cd	1.23 + 0.0153 cd	26.53 + 0.27a	9.03 + 0.04b
	T3	21.72 + 1.20a	43.64 + 0.62a	55.22 + 0.81a	1.27 + 0.0058bc	28.55 + 0.20a	9.76 + 0.18a
MD	CK	11.80 + 0.60 h	28.52 + 0.62 g	37.23 + 1.49f	1.31 + 0.0231b	15.93 + 0.85 fg	6.93 + 0.13f
	T1	16.48 + 0.46def	37.29 + 0.78 cd	44.52 + 1.02e	1.19 + 0.0058d	21.26 + 0.35bc	8.25 + 0.18 cd
	T2	15.61 + 0.84efg	36.48 + 0.56de	43.88 + 0.68e	1.20 + 0.0115 cd	20.46 + 0.22 cd	7.80 + 0.25de
	T3	19.04 + 0.45bc	41.27 + 1.02b	51.42 + 0.68b	1.24 + 0.0208 cd	22.99 + 0.27b	8.66 + 0.12bc
SD	CK	9.50 + 0.80i	21.15 + 1.56 h	30.51 + 0.78 g	1.45 + 0.0643a	13.73 + 0.69 g	6.23 + 0.40 g
	T1	14.75 + 1.05 fg	34.29 + 0.48ef	43.32 + 0.60e	1.26 + 0.0115bc	18.76 + 0.34de	7.42 + 0.13ef
	T2	14.22 + 0.84 g	32.47 + 0.80f	42.45 + 0.80e	1.31 + 0.0115b	17.48 + 0.23ef	7.01 + 0.09f
	T3	17.38 + 0.77cde	39.37 + 0.79bc	47.28 + 0.49d	1.20 + 0.0115 cd	20.52 + 0.18 cd	7.91 + 0.12de

[a] TN: total nitrogen; TP: total phosphorus; [b] ND: no drought stress; MD: moderate drought stress; SD: severe drought stress. [c] CK: control without bacteria; T1: *Pseudomonas lini*; T2: *Serratia plymuthica*; T3: *P. lini* + *S. plymuthica*. [d] Values are presented as the mean ± standard deviation ($n = 3$). Different lowercase letters indicate significant differences according to Ducan′ range test at the $P < 0.05$ level

soil aggregate stability, and relative water content were increased by 13.17%–27.87%, 54.80%–78.07%, and 22.41%–41.04%, respectively, compared to the control. The mixed inoculation resulted in a better RAS/RS ratio, soil aggregate stability, and relative water content than the use of single bacterial strains alone (Table 6).

Ethylene release content

The *acd*S mutant strains DT6s and DT8s unable to use ACC as sole nitrogen source were isolated and inoculated into the rhizosphere of jujube seedlings. Under drought stress conditions, inoculation of DT6 and DT8 significantly decreased the ethylene contents in the

Table 4 Antioxidant enzyme activity and malondialdehyde content of jujube leaves under different drought stress

Drought stress	Treatment	SOD (U·mg^{-1})	POD (U·mg^{-1})	MDA (μmol·g^{-1})
ND[a]	CK[b]	109.36 + 1.89f[c]	37.15 + 1.04f	17.12 + 0.23e
	T1	114.57 + 1.37f	42.13 + 1.27e	16.76 + 0.24ef
	T2	114.10 + 2.82f	41.63 + 0.49e	16.86 + 0.48ef
	T3	115.37 + 2.50f	43.34 + 0.71e	16.20 + 0.37f
MD	CK	130.72 + 3.06e	43.15 + 0.92e	21.64 + 0.34b
	T1	152.47 + 2.83c	52.40 + 1.46d	18.58 + 0.37d
	T2	149.52 + 3.12c	51.32 + 0.82d	18.04 + 0.23d
	T3	160.20 + 1.31b	56.76 + 0.41c	16.87 + 0.36ef
SD	CK	142.36 + 2.26d	51.26 + 1.05d	24.51 + 0.39a
	T1	165.29 + 2.72b	64.74 + 0.52b	19.72 + 0.35c
	T2	162.23 + 1.59b	63.15 + 0.64b	20.41 + 0.13c
	T3	174.39 + 2.54a	70.48 + 0.75a	17.89 + 0.23d

[a] ND: no drought stress; MD: moderate drought stress; SD: severe drought stress. [b] CK: control without bacteria; T1: *Pseudomonas lini*; T2: *Serratia plymuthica*; T3: *P. lini* + *S. plymuthica*. [c] Values are presented as the mean ± standard deviation ($n = 3$). Different lowercase letters indicate significant differences according to Ducan′ range test at the $P < 0.05$ level

Table 5 Plant hormone content in jujube leaves under different levels of drought stress

Drought stress	Treatment	IAA (μg·g^{-1}FW)	ABA (μg·g^{-1}FW)
ND[a]	CK[b]	50.34 + 1.02d[c]	31.59 + 2.80e
	T1	75.60 + 1.11b	30.45 + 2.23e
	T2	72.33 + 2.10b	30.09 + 2.20e
	T3	81.17 + 2.26a	29.13 + 1.90e
MD	CK	39.57 + 1.88e	53.39 + 2.20b
	T1	54.63 + 1.31d	46.51 + 2.29c
	T2	49.78 + 2.86d	47.25 + 1.40c
	T3	62.47 + 1.04c	40.47 + 1.56d
SD	CK	22.14 + 2.86 g	61.29 + 1.18a
	T1	37.75 + 1.70ef	54.48 + 2.94b
	T2	34.62 + 2.87f	55.60 + 1.15b
	T3	41.54 + 1.27e	47.80 + 1.82c

[a] ND: no drought stress; MD: moderate drought stress; SD: severe drought stress. [b] CK: control without bacteria; T1: *Pseudomonas lini*; T2: *Serratia plymuthica*; T3: *P. lini* + *S. plymuthica*. [c] Values are presented as the mean ± standard deviation ($n = 3$). Different lowercase letters indicate significant differences according to Ducan′ range test at the $P < 0.05$ level

leaves of jujube seedlings compared with control (non-inoculated treatment). However, the ethylene release amount in inoculated jujube seedlings with the *acd*S mutant strains were similar to control (Table 7).

Discussion

Bacterial ACC deaminase activities could help host plants alleviate drought stress by changing ACC to ammonia and α-ketobutyrate and consequently reducing the ethylene levels in plants (Kruasuwan and Thamchaipenet 2018). In this paper, we isolated eight ACC deaminase-producing bacterial strains with ACC deaminase activity levels that fluctuated between 34.55–234.98 μmol α-KA/(mg Pr·h). *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 showed the highest ACC deaminase activities among the eight strains, and under high levels of drought stress (−0.30 MPa), the ACC deaminase activities (DT6: 176.63 μmol α-KA/(mg Pr•h); DT8: 123.45 μmol α-KA/(mg Pr•h)) were only reduced by 33.04% and 38.83%, respectively, compared to normal water supply condition. Ali et al. (2014) reported that organisms with ACC deaminase activities of 20 nmol α-KA/(mg Pr•h) or higher could increase host plant resistance under stress conditions. Therefore, these two ACC deaminase-producing bacteria were chosen to further study their growth promoting characteristics under water shortage conditions. Inoculation with these two bacterial strains significantly increased the dry matter weight of jujube seedlings under drought stress conditions. This result was consistent with previous studies and indirectly demonstrated increased resistance to drought stress. In this paper, we isolated the *acd*S mutant strains of DT6 and DT8 which were unable to produce ACC deaminase. Inoculation with *acd*S mutant strains had no effect on reducing the release of stress induced ethylene under water shortage conditions. It demonstrated that the ethylene release amount in jujube seedlings could be related to ACC deaminase secreted by wild strains under stress conditions. ACC deaminase producing PGPR might help plants alleviate the damage of plants by reduction

Table 6 RAS/RT ratio, soil structure, and relative water content of jujube seedlings under serve stress condition

Drought stress	Treatment	RAS/RT ratio[c]	Aggregate stability (%)	Relative water content (%)
ND[a]	CK[b]	30.38 ± 2.35f[d]	37.14 ± 1.03d	51.73 ± 1.05c
	T1	33.06 ± 2.73ef	43.20 ± 0.95c	54.62 ± 0.89b
	T2	32.37 ± 1.72ef	42.50 ± 0.74c	53.43 ± 0.72bc
	T3	36.47 ± 1.41de	49.66 ± 0.97b	60.43 ± 1.25a
SD	CK	40.18 ± 1.18 cd	31.24 + 0.43e	37.79 ± 0.94e
	T1	48.49 ± 2.72ab	50.37 ± 0.71b	48.42 ± 1.22d
	T2	45.47 ± 2.10bc	48.36 ± 0.86b	46.26 ± 0.98d
	T3	51.38 ± 0.82a	55.63 ± 0.62a	53.30 ± 1.02c

[a] ND: no drought stress; MD: moderate drought stress; SD: severe drought stress. [b] CK: control without bacteria; T1: *Pseudomonas lini*; T2: *Serratia plymuthica*; T3: *P. lini* + *S. plymuthica*. [c] RAS/RT ratio: the ratio of root associated soil to root. [d] Values are presented as the mean ± standard deviation ($n = 3$). Different lowercase letters indicate significant differences according to Ducan′ range test at the $P < 0.05$ level

Table 7 Ethylene content (nmol g^{-1} FW h^{-1}) of jujube leaves under drought stress

Treatment	ND[a]	MD	SD
CK[b]	0.0853 ± 0.0037d[c]	0.1232 ± 0.0035b	0.1521 ± 0.0030a
DT6	0.0807 ± 0.0026d	0.0950 ± 0.0035c	0.1182 ± 0.0033b
DT8	0.0830 ± 0.0021d	0.1005 ± 0.0033c	0.1262 ± 0.0053b
DT6s	0.0849 ± 0.0026d	0.1210 ± 0.0029b	0.1501 ± 0.0026a
DT8s	0.0845 ± 0.0029d	0.1223 ± 0.0030b	0.1513 ± 0.0023a

[a] ND: no drought stress; MD: moderate drought stress; SD: severe drought stress. [b] CK: control without bacteria; DT6: *Pseudomonas lini*; DT8: *Serratia plymuthica*; DT6s: an *acd*S mutant strain of DT6; DT8s: an *acd*S mutant strain of DT8. [c] Values are presented as the mean ± standard deviation ($n = 3$). Different lowercase letters indicate significant differences according to Ducan′ range test at the $P < 0.05$ level

the concentration of ethylene. In addition, Niu et al. (2018) reported that *Pseudomonas* strains associated with millet showed high ACC deaminase activities in semiarid regions. Our results showed that the eight ACC deaminase-producing bacterial strains were isolated from jujube growing under arid conditions. ACC deaminase activities of these bacterial strains were significantly higher than those previously reported in *Pseudomonas* from other crops (157–972 (nmol α-KA/(mg Pr•h)) (Singh et al. 2015b). It was suggested that the environmental stresses of the host plant contributed to the induction of higher ACC deaminase activities in rhizosphere microorganisms (Niu et al. 2018). The rise in ACC deaminase activities was useful for reducing the stress-related ethylene levels and helping plants increase their resistance to stress. Therefore, isolates from habitats under stressful conditions provide a foundation for further use.

The distributions of ACC deaminase producing bacteria are different in the rhizosphere mainly depending on the plant species present (Gontia-Mishra et al. 2017). The genera in the rhizosphere of millet mainly include *Pseudomonas*, *Enterobacter*, and *Arthrobacter* (Niu et al. 2018). Gontia-Mishra reported that the predominant bacterial genera in the rhizosphere of wheat were *Acinetobacter*, *Klebsiella*, and *Enterobacter* (Gontia-Mishra et al. 2017). Our results showed that *Pseudomonas* was dominant in the jujube rhizosphere. It has been reported that most microbes may not be cultured by selective media (Joint et al. 2010). At present, some researchers evaluated the diversity of soil microbial communities by molecular biological methods and demonstrated that there were diverse microbes living in the plant rhizosphere (Gontia-Mishra et al. 2017). These microorganisms, especially PGPR contains many functional genes, such as IAA production (*iaaM*), nitrogen fixation (*nifU*), biosynthesis of spermidine (*speB*) and siderophore (*sbnA*), which facilitated plant growth and tolerance under stress conditions (Xiong et al. 2019). Their community and functional profiles varied with the stress degree (Liu et al. 2019b). Therefore, we speculated that there may be various drought tolerant microbes that cannot be cultured that are distributed in the jujube rhizosphere. Drought tolerance has been linked to many other species besides the isolated bacteria.

ACC deaminase producing PGPR alleviated drought stress by reducing ethylene, enhancing nutrient and water absorption, thus promoting plant growth. In this paper, we detected the effects of PGPR on the growth characteristics and physiological changes of jujube under drought stress conditions to determine how the bacterial strains helped the host plant alleviate stress. Our results showed that inoculation with *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 significantly increased the content of nitrogen and phosphorus in the leaves by 27.31–49.45% compared to uninoculated seedlings. The enhancement of nutrient contents might be associated with the good capabilities of phosphorus solubilization, nitrogen fixation, and IAA production in the two strains. In addition, it has been reported that drought stress obviously inhibits the nutrition uptake of plants. Under limiting conditions, plants adopt a kind of drought avoidance strategy that promotes the absorption of nutrients by inhibiting shoot growth while improving root dry weight and elongation (Prudent et al. 2015; Gupta and Pandey 2019). Such a drought avoidance strategy leads to an increase in the root/shoot ratio. In this paper, we found that PGPR decreased the root/shoot ratio in jujube trees compared to the uninoculated control. The results were consistent with the previous studies (Ali et al. 2017; Rocha et al. 2019) and indirectly demonstrated that PGPR inoculations effectively

alleviated drought stress. In addition, PGPR inoculation induced systemic tolerance processes and thus improved physical characteristics in jujube trees by increasing IAA, SOD, POD, and relative water content and reducing leaf MDA and ABA contents. According to the previous research results (Bal et al. 2013; Sarkar et al. 2018a), we deduced that overall enhancement of the growth characteristics and physiological changes of jujube seedlings might be associated with the reduction of ethylene and other PGP traits of these strains. The following paragraph elaborates the effect and mechanism of alleviating drought stress in jujube trees after inoculation with these strains.

Ethylene, as a very important phytohormone, widely existed in a serious of tissues and organs of plants. It plays a positive role in regulating plant growth at very low concentrations (Glick 2005; Dubois et al. 2018). However, the content of ethylene increased quickly under stress conditions and caused adverse impacts on plants, such as inducing chlorosis, inhibiting root and shoot growth, and restraining leaf expansion (Singh et al. 2015a, b). Our results showed that drought stress significantly increased the release of ethylene. Inoculation with ACC deaminase producing PGPR inhibited the ethylene biosynthesis in plants through secreting ACC deaminase, thereby alleviating the restraining function of growth in jujube caused by ethylene and improving the drought-resistance of jujube.

Generally, drought stress induced overproduction of reactive oxygen species (ROS) and destroyed normal cell metabolism by oxidative damage of membrane proteins, DNA, and lipids (Kaushal and Wani 2016). MDA plays an important role in membrane lipid peroxidation. Previous studies have shown that beneficial microbes can reduce the content of MDA, prevent the accumulation of ROS, increase activities of antioxidant enzymes and maintain plant growth under water deficit (Silambarasan et al. 2019). Our results showed that inoculation with *Pseudomonas lini*, *Serratia plymuthica*, or their mixture significantly reduced the MDA content of jujube seedlings under drought stress. This result suggested that inoculation with the three bacteria treatments decreased the detrimental effects of oxidative damage caused by ROS production under stress conditions. To remove excessive amounts of ROS, there is a ROS scavenging system that plants utilize to protect themselves. SOD and POD are the most important components of this scavenging system. SOD catalyzes the dismutation of O_2^- to oxygen and hydrogen peroxide (Saker and Oba 2018). POD plays an important role in catalyzing hydrogen peroxide to water and oxygen (Liu et al. 2019a). The ROS scavenging mechanisms of PGPR had been recently reported. Under environmental stress, excessive ROS along with MDA accumulated in plants by genes (e.g. *PgRboHD*; *PgFE*) transcription among cells. ROS accumulation primed transcription of antioxidant genes to scavenge ROS. Inoculation with PGPR increased expression of antioxidant genes, thus improved antioxidant enzyme activities (Sukweenadhi et al. 2018). The enhancements of these enzyme activities protected chloroplast from ROS and eliminated superoxide (Sarkar et al. 2018a, b). In this study, we found that the SOD and POD activities were significantly higher in inoculated jujube seedlings compared to the uninoculated seedlings, and the enzyme activities increased with increasing drought stress. Therefore, it is deduced that the three bacteria treatments improved the scavenging activities of jujube by regulating the expression of antioxidant genes and enhancing SOD and POD activities under drought stress conditions to reduce MDA content.

Under drought stress conditions, PGPR strains have been reported to produce exopolysaccharides (EPS). These high-molecular weight carbohydrate compounds play a significant role in promoting plant growth through the formation of hydrophilic biofilms, which provide a microenvironment that holds water and dries more slowly around the rhizosphere, thus protecting bacteria from drying (Forni et al. 2017). Microbial EPS production regulated the plant growth promoting attributes, such as nitrogen fixation, phosphorus solubilization, and hormone secretion, and thus improved the drought tolerance of the plant (Mukherjee et al. 2019). It was reported that EPS-producing strains increased the RAS/RT ratio and macroaggregate stability, which enhanced water and nutrient uptake from the soil reserve (Vardharajula et al. 2011; Kaushal 2019). In the present study, EPS-producing *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 significantly increased the RAS/RT ratio, soil aggregation stability and leaf relative water content and thus improved plant growth under drought stress conditions. Therefore, these data indicated that EPS producing bacterial strains also could increase water availability in plants by the following way: aggregation of the rhizosphere soil. Better aggregation could help plants absorb more nutrients and water from rhizosphere soil, alleviating the damage to plants caused by drought stress. In addition, it was reported that the

effects of EPS on keeping higher water potential and alleviating plant drought stress mainly relied on their component, structure, and production (Moshabaki Isfahani et al. 2018; Tahir et al. 2019; Mukherjee et al. 2019). These factors varied with bacterial species, growth phase, and stress conditions (Costa et al. 2018). Our study demonstrated that *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 could synthesize EPSs and protected their functioning under drought stress. However, the structure, component, and amounts of EPSs under different drought stresses should be detected to help these two bacterial strains function better.

Phytohormones are critical for the process of plant adaptation to various stress conditions such as drought, high salinity, and cold (Potters et al. 2007). Many studies have reported that beneficial microbes are able to modulate the production of phytohormones, such as IAA and ABA in plants under stress (Vives-Peris et al. 2018; Zubair et al. 2019; Jochum et al. 2019). Our results showed that inoculation with *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 significantly induced the accumulation of IAA in the leaves of jujube seedlings under drought conditions. These findings demonstrated the involvement of IAA in the adaptation to drought. Timmusk et al. reported that inoculation with IAA-producing *Bacillus thuringiensis* significantly increased lateral root density, length, and root hair density of wheat under conditions of drought stress (Timmusk et al. 2014). Under stress conditions, PGPR strains might upregulate the content of endogenous IAA in plants, which altered root structure and increased lateral roots. These ways improved the water and nutrient uptake efficiencies of plant (Li et al. 2017; Jochum et al. 2019). In the present study, the two PGPR strains, *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 detected in the rhizosphere had strong capacities to synthesize IAA (*Pseudomonas lini* DT6: 30.82 ± 0.34 $\mu g \cdot mL^{-1}$; *Serratia plymuthica* DT8: 45.75 ± 0.50 $\mu g \cdot mL^{-1}$), and they formed stable colonies on the root surface of jujube. In addition, accumulation of IAA content in inoculated plants could alter ROS metabolism and metabolic homeostasis (Sharma et al. 2018). Thus, we speculated that exogenous IAA produced by microorganisms might induce the newly synthesized IAA in jujube seedlings. The newly synthesized IAA combined with endogenous IAA served as a signal molecule that not only regulates root growth and leads to increased water acquisition and nutrient uptake but also strengthens SOD and POD enzyme activities and rapidly removes excessive ROS, ultimately alleviating stress damage. However, this speculation needs to be confirmed in further studies.

In addition to IAA, ABA is commonly known as an important stress hormone that accumulates in plants under drought stress. It was able to control stomatal closure and stress signal transduction pathways in plants during water deficit (Haworth et al. 2018). The decrease in stomatal conductance might be useful for the plants to reduce transpiration and thus alleviate drought stress. In addition, it has been demonstrated previously that ACC-deaminase producing bacteria could inhibit ethylene biosynthesis. The amount of ethylene was correlated with ABA levels. Therefore, ethylene reduction resulted in the decline of ABA level in plants (Vives-Peris et al. 2018). Our results also showed that bacterial strain inoculation significantly reduced the ABA concentration in the leaves compared to that in the uninoculated plants under drought stress conditions. Similar results were observed in tomato (Belimov et al. 2015), citrus (Vives-Peris et al. 2018) and maize (Skz et al. 2018). These results suggested that stress was lower in inoculated plants under drought conditions, and this is circumstantial evidence that inoculation of the two strains may effectively lessen drought stress.

Above all, inoculation with *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 induced systemic effects including physiological and biochemical changes in plants to promote plant growth under drought stress conditions. It was reported that under stress conditions, PGPR strains activated stress management in plants mainly by two ways: (1) regulate gene kinds or expression involved in scavenging ROS enzymes, biosynthesis of ethylene, catalase, superoxide dismutase, peroxidase and signaling of endogenous phytohormone in plants (Skz et al. 2018; Carlson et al. 2019); (2) reprogramming metabolic pathways (Abd El-Daim et al. 2019). Therefore, in order to acquire a deep understand of the drought resistant mechanism about the two strains, the molecular ways causing plant physiological changes induced by them need to be studied further.

Mixed inoculations had better effects on promoting plant growth compared to single inoculations (Boyer et al. 2015; Castanheria et al. 2017; Zafar-ul-Hye et al. 2019). Our results were agreement with these findings and showed that the most pronounced beneficial effects on jujube seedling growth and drought tolerance enhancement were observed in

the co-inoculation of *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8. This is mainly due to the synergistic effects between microorganisms, which not only enhanced their colonizing abilities in stress environments but also improved their abilities to obtain resources from plants and their environments by secreting more organic substances, such as exopolysaccharides and hormones, and indirectly enhanced plant growth survival and stress tolerance. Therefore, mixed inoculation may be beneficial for biofertilizer application. Although this study demonstrated that the two bacterial strains *Pseudomonas lini* DT6 and *Serratia plymuthica* DT8 play an important role in promoting plant growth and stress tolerance in jujube by pot experiments, the use of these strains in the field will be studied in the future to detect their effects and stability under those conditions.

Conclusions

The present study indicates that there were a variety of rhizobacteria with high drought tolerance and plant growth promoting traits that colonized the rhizosphere soil of jujube in arid regions. The majority of drought-tolerant bacteria in the jujube rhizosphere belong to *Pseudomonas*. These strains were isolated and evaluated for their effects on plant growth under drought stress. Inoculation tests showed that the bacterial strains *P. lini* or *S. plymuthica* could alleviate drought stress damage and improve plant survival by enhancing plant growth, regulating IAA and ABA contents and inducing the ROS scavenging system. In addition, these two strains produced EPSs, which improved soil structure and colonization abilities. The successful application of the PGPR strains mainly depends on abiotic and biotic factors. Therefore, further field studies should be undertaken to evaluate the inoculation effects of these two strains on alleviating jujube drought stress before they can serve as new bioinoculants for use in agriculture, especially in arid regions.

Acknowledgements This study was financially supported by Key Science and Technology Program of Shaanxi Province (2016NY-169), National Key Research and Development Program of China (2016YFC0501706-1), China Postdoctoral Science Foundation (2016M60082), Tang Scholar Program of Northwest A&F University, and Fundamental Research Funds for the Central Universities (2452018132).

Author contributions Xuan Yu and Min Zhang conceived and designed the experiment; Min Zhang and Lin Yang completed the experiment; Ruqian Hao, Xiaoxiong Bai and Ying Wang analyzed the experimental datas; Xuan Yu and Min Zhang wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Abd El-Daim IA, Bejai S, Meijer J (2019) *Bacillus velezensis* 5113 induced metabolic and molecular reprogramming during abiotic stress tolerance in wheat. Sci Rep-UK 9:16282. https://doi.org/10.1038/s41598-019-52567-x

Abedi T, Pakniyat H (2010) Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). Czech J Genet Plant Breed 46:27–34. https://doi.org/10.17221/67/2009-CJGPB

Ali SZ, Sandhya V, Rao LV (2014) Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent *Pseudomonas* sp. Ann Microbiol 64: 493–502. https://doi.org/10.1007/s13213-013-0680-3

Ali L, Khalid M, Asghar HN, Asgher M (2017) Scrutinizing of rhizobacterial isolates for improving drought resilience in maize (*Zea mays*). Int J Agric Biol 19:1054–1064. https://doi.org/10.17957/IJAB/15.0387

Aslantas R, Cakmakci R, Sahin F (2007) Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. Sci Hortic 111:371–377. https://doi.org/10.1016/j.scienta.2006.12.016

Bal HB, Nayak L, Das S, Adhya TK (2013) Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil 366(1–2):93–105. https://doi.org/10.1007/s11104-012-1402-5

Bartoli F, Burtin G, Herbillon AJ (1991) Disaggreration and clay dispersion of oxisols: Na resin, a recommended methodology. Geoderma 49:301–317. https://doi.org/10.1016/0016-7061(91)90082-5

Batista BD, Lacava PT, Ferrari A, Teixeira-Silva NS, Bonatelli ML, Tsui S, Mondin M, Kitajima EW, Pereira JO, Azevedo JL, Quecine MC (2018) Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. Microbiol Res 206:33–42. https://doi.org/10.1016/j.micres.2017.09.007

Belimov AA, Dodd IC, Safronova VI, Shaposhnikov AI, Azarova TS, Makarova NM, Davies WJ, Tikhonovich IA (2015) Rhizobacteria that produce auxins and contain 1-aminocyclopropane −1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). Ann Appl Biol 167:11–25. https://doi.org/10.1111/aab.12203

Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some larger consequences of minor changes in conditions. Anal Biochem 161:559–566. https://doi.org/10.1016/0003-2697(87)90489-1

Boyer LR, Brain P, Xu MN, Jeffries P (2015) Inoculation of drought-stresses strawberry with a mixed inoculum of two arbuscular mycorrhizal fungi: effects on population dynamics of fungal species in roots and consequential plant tolerance to water deficiency. Mycorrhiza 25:215–227. https://doi.org/10.1007/s00572-014-0603-6

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Calvo-Polanco M, Sánchez-Romera B, Aroca R, Asins MJ, Declerck S, Dodd IC, Martinez-Andujar C, Albacete A, Ruiz-Lozano JM (2016) Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato. Environ Exp Bot 131:47–57. https://doi.org/10.1016/j.envexpbot.2016.06.015

Carlson R, Tugizimana F, Steenkamp PA, Dubery IA, Hassen AI, Labuschagne N (2019) Rhizobacteria-induced systemic tolerance against drought stress in *Sorghum bicolor* (L.) Moench. Microbiol Res 19:30837–30837. https://doi.org/10.1016/j.micres.2019.126388

Castanheria NL, Dourado AC, Pais I, Semedo J, Scotti-Campos P, Borges N, Carvalho G, Crespo MTB, Fareleira P (2017) Colonization and beneficial effects on annual ryegrass by mixed inoculation with plant growth promoting bacteria. Microbiol Res 198:47–55. https://doi.org/10.1016/j.micres.2017.01.009

Cattelan AJ, Hartel PG, Fuhrmann JJ (1999) Screening for plant growth-promoting rhizobacteria to promote early soybean growth. Soil Sci Soc Am J 63:1670–1680. https://doi.org/10.2136/sssaj1999.6361670x

Cedeno-Garcia GA, Gerding M, Moraga G, Inostroza L, Fischer S, Sepulveda-Caamano M, Oyarzua P (2018) Plant growth promoting rhizobacteria with ACC deaminase activity isolated from Mediterranean dryland areas in Chile: effects on early nodulation in alfalfa. Chil J Agr Res 78:360–369. https://doi.org/10.4067/S0718-58392018000300360

Chandra D, Srivastava R, Glick BR, Sharma AK (2018a) Drought-tolerant *Pseudomonas* spp. improve the growth performance of finger millet (*Eleusine coracana* (L.) Gaertn.) under non-stressed and drought-stressed conditions. Pedosphere 28:227–240. https://doi.org/10.1016/51002-0160(18)60013-X

Chandra P, Tripathi P, Chandra A (2018b) Isolation and molecular characterization of plant growth-promoting *Bacillus* spp. and their impact on sugarcane (*Saccharum* spp. hybrids) growth and tolerance towards drought stress. Acta Physiol Plant 40:1–5. https://doi.org/10.1007/s11738-018-2770-0

Chandra R, Amit, Ghosh UK (2019) Effects of various abiotic factors on biomass growth and lipid yield of *Chlorella minutissima* for sustainable biodiesel production. Environ Sci Pollut Res Int 26:3848–3861. https://doi.org/10.1007/s11356-018-3696-1

Chen DY, Wang YK, Wang X, Nie ZY, Gao ZY, Zhang LL (2016) Effects of branch removal on water use of rain-fed jujube (*Ziziphus jujuba* mill.) plantations in Chinese semiarid loess plateau region. Agric Water Manag 178:258–270. https://doi.org/10.1016/j.agwat.2016.10.010

Costa OYA, Raaijmakers JM, Kuramae EE (2018) Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front Microbiol 9:1–14. https://doi.org/10.3389/fmicb.2018.01636

Cui YX, Fang LC, Guo XB, Wang X, Zhang YJ, Li PF, Zhang XC (2018) Ecoenzymatic stoichiometry and microbial nutrient limitation in rhizosphere soil in the arid area of the northern loess plateau, China. Soil Biol Biochem 116:11–21. https://doi.org/10.1016/j.soilbio.2017.09.025

Danish S, Zafar-ul-Hye M (2019) Co-application of ACC-deaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress. Sci Rep-UK 9:1–13. https://doi.org/10.1038/s41598-019-42374-9

Day JM, Döbereiner J (1976) Physiological aspects of N_2-fixation by a *Spirillum* from *Digitaria* roots. Soil Biol Biochem 8:45–50. https://doi.org/10.1016/0038-0717(76)90020-1

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356. https://doi.org/10.1021/ac60111a017

Dubois M, Lisa VDB, Inzé D (2018) The pivotal role of ethylene in plant growth. Trends Plant Sci 23:311–323. https://doi.org/10.1016/j.tplants.2018.01.003

Forni C, Duca D, Glick BR (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil 410:335–356. https://doi.org/10.1007/s11104-016-3007-x

Garcia JE, Maroniche G, Creus C, Suarez-Rodriguez R, Ramirez-Trujillo JA, Groppa MD (2017) In vitro PGPR properties and osmotic tolerance of different *Azospirillum* native strains and their effects on growth of maize under drought stress. Microbiol Res 202:21–29. https://doi.org/10.1016/j.micres.2017.04.007

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7. https://doi.org/10.1016/j.femsle.2005.07.030

Glick BR, Karaturovíc DM, Newell PC (1995) A novel procedure for rapid isolation of plant growth promoting pseudomonads. Can J Microbiol 41:533–536. https://doi.org/10.1139/m95-070

Gontia-Mishra I, Sapre S, Kachare S, Tiwari S (2017) Molecular diversity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing PGPR from wheat (*Triticum aestivum* L.) rhizosphere. Plant Soil 414:213–227. https://doi.org/10.1007/s11104-016-3119-3

Grodzinski B, Boesel I, Horton RF (1982) Ethylene release from leaves of *Xanthium strumarium* L. and *Zea mays* L. J Exp Bot 33:344–354. https://doi.org/10.1093/jxb/33.2.344

Gupta S, Pandey S (2019) ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. Front Microbiol 10:1–17. https://doi.org/10.3389/fmicb.2019.01506

Haworth M, Marino G, Cosentino SL, Brunetti C, De Carlo A, Avola G, Riggi E, Loreto F, Centritto M (2018) Increased free abscisic acid during drought enhances stomatal sensitivity and modifies stomatal behaviour in fast growing giant

reed (*Arundo donax* L.). Environ Exp Bot 147:116–124. https://doi.org/10.1016/j.envexpbot.2017.11.002

Huang XF, Zhou DM, Lapsansky ER, Reardon KF, Guo JH, Andales MJ, Vivanco JM, Manter DK (2017) *Mitsuaria* sp. and *Burkholderia* sp. from *Arabidopsis* rhizosphere enhance drought tolerance in *Arabidopsis thaliana* and maize (*Zea mays* L.). Plant Soil 419:523–539. https://doi.org/10.1007/s11104-017-3360-4

Islam F, Yasmeen T, Ali Q, Ali S, Arif MS, Hussain S, Rizvi H (2014) Influence of *Pseudomonas aeruginosa* as PGPR on oxidative stress tolerance in wheat under Zn stress. Ecotoxicol Environ Saf 104:285–293. https://doi.org/10.1016/j.ecoenv.2014.03.008

Ji Q, Wang DW, Zhou J, Xu YL, Shen BQ, Zhou F (2019) Genome-wide characterization and expression analyses of the MYB superfamily genes during development stages in Chinese jujube. PEER J 7:1–28. https://doi.org/10.7717/peerj.6353

Jia JB, Li SJ, Cao X, Li H, Shi WG, Polle A, Liu TX, Peng CH, Luo ZB (2015) Physioloical and transcriptional regulation in poplar roots and leaves during acclimation to high temperature and drought. Physiol Plant 157:38–53. https://doi.org/10.1111/ppl.12400

Jochum MD, McWilliams KL, Borrego EJ, Kolomiets MV, Niu GH, Pierson EA, Jo YK (2019) Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. Front Microbiol 10:1–9. https://doi.org/10.3389/fmicb.2019.02106

Joint I, Mühling M, Querellou J (2010) Culturing marine bacteria-an essential prerequisite for biodiscovery. Microb Biotechnol 3:564–575. https://doi.org/10.1111/j.1751-7915.2010.00188.x

Kaushal M (2019) Microbes in cahoots with plants: MIST to hit the Jackpot of agriculture productivity during drought. Int J Mol Sci 20:1769. https://doi.org/10.3390/ijms20071769

Kaushal M, Wani SP (2016) Rhizobacterial-plant interactions: strategies ensuring plant growth promotion under drought and salinity stress. Agric Ecosyst Environ 231:68–78. https://doi.org/10.1016/j.agee.2016.06.031

Khan N, Bano A, Shahid MA, Nasim W, Babar MDA (2018a) Interaction between PGPR and PGR for water conservation and plant growth attributes under drought condition. Biologia 73:1083–1098. https://doi.org/10.2478/s11756-018-0127-1

Khan N, Zandi P, Ali S, Mehmood A, Shahid MA (2018b) Impact of salicylic acid and PGPR on the drought tolerance and phytoremediation potential of *Helianthus annus*. Front Microbiol 9:1–15. https://doi.org/10.3389/fmicb.2018.02507

Khan N, Bano A, Zandi P (2018c) Effects of exogenously applied plant growth regulations in combination with PGPR on the physiology and root growth of chickpea (*Cicer arietinum*) and their role in drought tolerance. J Plant Interact 13:239–247. https://doi.org/10.1080/17429145.2018.1471527

Kruasuwan W, Thamchaipenet A (2018) 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing endophytic diazotrophic *Enterobacter* sp EN-21 modulates salt-stress response in sugarcane. J Plant Growth Regul 37:849–858. https://doi.org/10.1007/s00344-018-9780-4

Li HS, Lei P, Peng X, Li S, Xu H, Xu ZQ, Feng XH (2017) Enhanced tolerance to salt stress in canola (*Brassica napus* L.) seedlings inoculated with the halotolerant *Enterbacter cloacae* HSNJ4. Appl Soil Ecol 119:26–34. https://doi.org/10.1016/j.apsoil.2017.05.033

Liu J, Hasanuzzaman M, Wen HL, Zhang J, Peng T, Sun HW, Zhao QZ (2019a) High temperature and drought stress cause abscisic acid and reactive oxygen species accumulation and suppress seed germination growth in rice. Protoplasma 256:1217–1227. https://doi.org/10.1007/s00709-019-01354-6

Liu K, Cai MM, Hu CX, Sun XC, Cheng Q, Jia W, Yang T, Nie M, Zhao XH (2019b) Selenium (se) reduces Sclerotinia stem rot disease incidence of oilseed rape by increasing plant se concentration and shifting soil microbial community and functional profiles. Environ Pollut 254:113051. https://doi.org/10.1016/j.envpol.2019.113051

Malik DK, Sindhu SS (2011) Production of indole acetic acid by *Pseudomonas* sp.: effect of coinoculation with *Mesorhizobium* sp. *Cicer* on nodulation and plant growth of chickpea (*Cicer arietinum*). Physiol Mol Biol Plants 17:25–32. https://doi.org/10.1007/s12298-010-0041-7

Martins SJ, Rocha GA, de Melo HC, Georg RD, Ulhoa CJ, Dianese ED, Oshiquiri LH, da Cunha MG, da Rocha MR, de Araujo LG, Vaz KS, Dunlap CA (2018) Plant-associated bacteria mitigate drought stress in soybean. Environ Sci Pollut Res Int 25:13676–13686. https://doi.org/10.1007/s11356-018-1610-5

Michel BE, Kaufmann MR (1973) The osmotic potential of polyethylene glycol 6000. Plant Physiol 51:914–916. https://doi.org/10.1104/pp.51.5.914

Misra S, Dixit VK, Mishra SK, Chauhan PS (2019) Demonstrating the potential of abiotic stress-tolerant *Jeotgalicoccus huakuii* NBRI 13E for plant growth promotion and salt stress amenlioration. Ann Microbiol 69:419–434. https://doi.org/10.1007/s13213-018-1428-x

Moshabaki Isfahani F, Tahmourespour A, Hoodaji M, Ataabadi M, Mohammadi A (2018) Characterizing the new bacterial isolates of high yielding exopolysaccharides under hypersaline conditions. J Clean Prod 185:922–928. https://doi.org/10.1016/j.jclepro.2018.03.030

Mukherjee P, Mitra A, Roy M (2019) *Halomonas* rhizobacteria of *Avicennia marina* of India Sundarbans promote rice growth under saline and heavy metal stresses through exopolysaccharide production. Front Microbiol 10:1–18. https://doi.org/10.3389/fmicb.2019.01207

Niu XG, Song LC, Xiao YN, Ge WD (2018) Drought-tolerant plant growth-promoting rhizobacteria associated with foxtail millet in a semi-arid agroecosystem and their potential in alleviating drought stress. Front Microbiol 8:1–11. https://doi.org/10.3389/fmicb.2017.02580

Pan D, Song YQ, Dyck M, Gao XD, Wu PT, Zhao XN (2017) Effect of plant cover type on soil water budget and tree photosynthesis in jujube orchards. Agric Water Manag 184:135–144. https://doi.org/10.1016/j.agwat.2017.01.009

Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol Plant 118:10–15. https://doi.org/10.1034/j.1399-3054.2003.00086.x

Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen MAK (2007) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12:98–105. https://doi.org/10.1016/j.tplants.2007.01.004

Prudent M, Salon C, Souleimanov A, Emery RJN, Smith DL (2015) Soybean is less impacted by water stress using

Bradyrhizobium japonicum and thuricin-17 from *Bacillus thuringiensis*. Agron Sustain Dev 35:749–757. https://doi.org/10.1007/s13593-014-0256-z

Qin S, Zhang YJ, Yuan B, Xu PY, Xing P, Wang J, Jiang JH (2014) Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte *Limonium sinense* (Girard) Kuntze and evaluating their plant growth-promoting activity under salt stress. Plant Soil 374: 753–766. https://doi.org/10.1007/s11104-013-1918-3

Ravanbakhsh M, Sasidharan R, Voesenek LACJ, Kowalchuk GA, Jousset A (2018) Microbial modulation of plant ethylene signaling: ecological and evolutionary consequences. Microbiome 6:52. https://doi.org/10.1186/s40168-018-0436-1

Rocha I, Ma Y, Vosatka M, Freitas H, Oliveira RS (2019) Growth and nutrition of cowpea (*Vigna unguiculata*) under water deficit as influenced by microbial inoculation via seed coating. J Agron Crop Sci 205:447–459. https://doi.org/10.1111/jac.12335

Saker U, Oba S (2018) Catalase, superoxide dismutase and ascorbate-gliutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. Sci Rep-UK 8:1–12. https://doi.org/10.1038/s41598-018-34944-0

Sandhya V, Ask Z, Grover M, Reddy G, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biol Fertil Soils 46:17–26. https://doi.org/10.1007/s00374-009-0401-z

Sarkar A, Ghosh PK, Pramanik K, Mitra S, Soren T, Pandey S, Mondal MH, Maiti TK (2018a) A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. Res Microbiol 169:20–32. https://doi.org/10.1016/j.resmic.2017.08.005

Sarkar J, Chakraborty B, Chakraborty U (2018b) Plant growth promoting rhizobacteria protect wheat plants against temperature stress through antioxidant signaling and reducing chloroplast and membrane injury. J Plant Growth Regul 37: 1396–1412. https://doi.org/10.1007/s00344-018-9789-8

Sharma L, Dalal M, Verma RK, Kumar SVV, Yadav SK, Pushkar S, Kushwaha SR, Bhowmik A, Chinnusamy V (2018) Auxin protects spikelet fertility and grain yield under drought and heat stresses in rice. Environ Exp Bot 150:9–24. https://doi.org/10.1016/j.envexpbot.2018.02.013

Sharp RE, Hsiao TC, Silk WK (1990) Growth of the maize primary root at low water potentials: II. Role of growth and deposition of hexose and potassium in osmotic adjustment. Plant Physiol 93:1337–1346. https://doi.org/10.1104/pp.93.4.1337

Silambarasan S, Logeswari P, Valentine A, Cornejo P (2019) Role of *Curtobacterium herbarum* strain CAH5 on aluminum bioaccumulation and enhancement of *Lactuca sativa* growth under aluminum and drought stresses. Ecotoxicol Environ Saf 183:109573. https://doi.org/10.1016/j.ecoenv.2019.109573

Singh RP, Jha P, Jha PN (2015a) The plant-growth-promoting bacterium *Klebsiella* sp: SBP-8 confers induced systemic tolerance in wheat (*Triticum aestivum*) under salt stress. J Plant Physiol 184:57–67. https://doi.org/10.1016/j.jplph.2015.07.002

Singh RP, Shelke GM, Kumar A, Jha PN (2015b) Biochemistry and genetics of ACC deaminase: a weapon to "stress ethylene" produced in plants. Front Microbiol 6:937. https://doi.org/10.3389/fmicb.2015.07255

Skz A, Vardharajula S, Vurukonda SSKP (2018) Transcriptomic profiling of maize (*Zea mays* L.) seedlings in response to *Pseudomonas putida* stain FBKV2 inoculation under drought stress. Ann Microbiol 68:331–349. https://doi.org/10.1007/S13213-018-1314-1

Sukweenadhi J, Balusamy SR, Kim YJ, Lee CH, Kim YJ, Koh SC, Yang DC (2018) A growth-promoting bacteria, *Paenibacillus yonginensis* DCY 84T enhanced salt stress tolerance by activating defense-related systems in Panax ginseng. Front Plant Sci 9:1–17. https://doi.org/10.3389/fpls.2018.00813

Tahir M, Khalid U, Khan MB, Shahid M, Ahmad I, Akram M, Ijaz M, Hussain M, Farooq ABU, Naeem MA, Ahmad N (2019) Auxin and 1-aminocyclopropane-1-carboxylate deaminase activity exhibiting rhizobacteria improved maize quality and productivity under drought conditions. Int J Agric Biol 21:943–954. https://doi.org/10.17957/IJAB/15.0979

Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Kannaste A, Behers L, Nevo E, Seisenbaeva G, Stenstrom E, Niinemets U (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PLoS One 9:1–13. https://doi.org/10.1371/journal.pone.0096086

US Department of Agriculture (2010) Keys to soil taxonomy, 11th edn. U.S. Government Printing Office

Vanderstraeten L, Van Der Straeten D (2017) Accumulation and transport of 1-Aminocyclopropane-1-carboxylic acid (ACC) in plants: current status, considerations for future research and agronomic applications. Front Plant Sci 8:1–18. https://doi.org/10.3389/fpls.2017.00038

Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6:1–14. https://doi.org/10.1080/17429145.2010.535178

Vives-Peris V, Gomez-Cadenas A, Perez-Clemente RM (2018) Salt stress alleviation in citrus plants by plant growth-promoting rhizobacteria *Pseudomonas putida* and *Novosphingobium* sp. Plant Cell Rep 37:1557–1569. https://doi.org/10.1007/s00299-018-2328-z

Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24. https://doi.org/10.1016/j.micres.2015.12.003

Wang JF, Kang SZ, Li FS, Zhang FC, Li ZJ, Zhang JH (2008) Effects of alternate partial root-zone irrigation on soil microorganism and maize growth. Plant Soil 1-2:45–52. https://doi.org/10.1007/s11104-007-9453-8

Wang BX, Seiler JR, Mei CS (2016) A microbial endophyte enhanced growth of swichgrass under two drought cycles improving leaf level physiology and leaf development. Environ Exp Bot 122:100–108. https://doi.org/10.1016/j.envexpbot.2015.09.004

Watanabe FS, Olsen SR (1965) Test of an ascorbic acid method for determining phosphorous in water and $NaHCO_3$ extracts from soil. Soil Sci Soc Am J 29:677–678. https://doi.org/10.2136/sssaj1965.03615995002900060025x

Williams MA, Rice CW (2007) Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. Appl Soil Ecol 3:535–545. https://doi.org/10.1016/j.apsoil.2006.09.014

Xiong YW, Gong Y, Li XW, Chen P, Ju XY, Zhang CM, Yuan B, Lv ZP, Xing K, Qin S (2019) Enhancement of growth and salt tolerance of tomato seedlings by a natural halotolerant actinobacterium *Glutamicibacter halophytocola* KLBMP 5180 isolated from a coastal halophyte. Plant Soil 445:307–322. https://doi.org/10.1007/s11104-019-04310-8

Yuan J, Wu JC, Zhao ML, Wen T, Huang QW, Shen QR (2018) Effect of phenolic acids from banana root exudates on root colonization and pathogen suppressive properties of *Bacillus amyloliquefaciens* NJN-6. Biol Control 125:131–137. https://doi.org/10.1016/j.biocontrol.2018.05.016

Zafar-ul-Hye M, Danish S, Abbas M, Ahmad M, Munir TM (2019) ACC deaminase producing PGPR *Bacillus amyloliquefaciens* and *Agrobacterium fabrum* along with biochar improve wheat productivity under drought stress. Agronomy-Basel 9:1–16. https://doi.org/10.3390/agronomy9070343

Zhang XH, Lang DY, Zhang EH (2016) Effects of soil sterilization on growth of *Angelica sinensis* plant and soil microbial populations in a continuous mono-cropping soil. Int J Agric Biol 18:458–463. https://doi.org/10.17957/IJAB/15.0118

Zhou WJ, Leul M (1998) Uniconazole-induced allevation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. Plant Growth Regul 26:41–47. https://doi.org/10.1023/a:1006004921265

Zubair M, Hanif A, Farzand A, Sheikh TMM, Khan AR, Suleman M, Ayaz M, Gao XW (2019) Genetic screening and expression analysis of psychrophilic *Bacillus* spp. reveal their potential to alleviate cold stress and modulate phytohormones in wheat. Microorganisms 7:1–26. https://doi.org/10.3390/microorganisms7090337