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# Effects of *Bacillus amyloliquefaciens* QST713 on Photosynthesis and Antioxidant Characteristics of Alfalfa (*Medicago sativa* L.) under Drought Stress

**Lingjuan Han [1], Miaoling Zhang [1], Lixia Du [1], Li Zhang [2] and Bin Li [2,*]**

[1] College of Grassland Science, Shanxi Agricultural University, Taigu 030801, China
[2] Collaborative Innovation Center for Improving Quality and Increase Profits of Protected Vegetables in Shanxi, College of Horticulture, Shanxi Agricultural University, Taigu 030801, China
* Correspondence: libin080@sxau.edu.cn

**Abstract:** Drought stress is a prevalent abiotic stress that adversely affects multiple physiological processes in plants, especially their photosynthetic capacity. Application of plant growth–promoting rhizobacteria (PGPR) has been considered as an eco-friendly strategy to ameliorate the deleterious effects of drought stress on plants. The present study was carried out to investigate the effects of *Bacillus amyloliquefaciens* QST713 on plant growth, leaf relative water content (RWC), photosynthesis processes, photosynthetic pigment content and antioxidant enzyme activities in two alfalfa varieties, Galalxie Max (drought-tolerant) and Saidi 7 (drought-sensitive) under drought conditions. The results showed that drought stress significantly declined plant biomass production, RWC, photosynthetic pigment content (Chl a, Chl b and carotenoids) and photosynthetic gas exchange parameters (transpiration rate (Tr), stomatal conductance (Gs) and intercellular $CO_2$ concentration (Ci)), whereas it enhanced the enzymatic activity of peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) in both cultivars. In contrast, the inoculation of the bacillus strain QST713 was more effective on plant growth, showing higher plant biomass production compared to the non-inoculated plants under drought stress. Moreover, the application of QST713 significantly promoted the content of RWC, the accumulation of chlorophyll content and the activities of antioxidant enzymes as well as enhanced the photosynthetic capacity of alfalfa seedlings under drought stress. These results suggest that QST713 could be considered as a promising bio-inoculant for plants exposed to environmental stresses.

**Keywords:** alfalfa; drought stress; *Bacillus amyloliquefaciens* QST713; growth; photosynthesis; antioxidant



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## 1. Introduction

Drought is one of the primary abiotic stresses, constraining plant growth and development and resulting in the decline of agriculture productivity in arid and semiarid areas [1]. It has seen increased frequency and severity as a result of global warming [2]. Water stress has adverse effects on plants by changing water relations, decreasing nutrient uptake and reducing the rate of net photosynthetic and transpiration [3]. It also boosts the generation of reactive oxygen species (ROS), including superoxide anion radicals ($O_2.^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals (OH), etc., which affects the ambient redox regulatory state of plant cells and causes various physiological and biological changes in plants [4]. In such conditions, plants develop the antioxidant enzymes (SOD, POD, CAT and APX) that participate in cell defense against active oxygen injury in enzyme protection systems [5]. Furthermore, previous studies have indicated that the accumulation of ROS in chloroplasts could cause inhibition of photosynthesis processes [6,7].

Application of plant growth–promoting rhizobacteria (PGPR), an eco-friendly strategy employed to alleviate the deleterious effects of drought stress on plants, may contribute to the sustainability and cost-effectiveness of agricultural practices [3,8]. PGPR

can enhance plant growth under abiotic stress in two different ways: directly and indirectly. The direct mechanisms are as follows: (i) producing phytohormones, such as gibberellins (GAs), abscisic acid (ABA), cytokinins and indole-3-acetic acid (IAA); (ii) secreting 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme to reduce the precursor of ethylene; (iii) inducing exopolysaccharide (EPS) production, which involves biofilm formation and enhances the rhizosphere soil aggregation that leads to increased water and nutrient availability for plants; (iv) fixing atmospheric nitrogen for plants; (v) increasing the uptake of other nutrients such as phosphorous, zinc and potassium; and (vi) supplying Fe to plants through the production of siderophores [3,9,10]. The indirect mechanisms include: (i) promoting photosynthesis rates in plants with the production of phytohormone cytokinin; (ii) improving signal transduction; and (iii) enhancing immune responses for optimal growth [9,11]. Previously, *Pseudomonas azotoformans* FAP5 was shown to produce EPS, IAA, bioflm, ACC deaminase and solubilized tricalcium phosphate, which play considerable roles in resisting abiotic stress in plants [12]. Mariotti et al. [13] also found that *Azospirillum baldaniorum* Sp245 could synthesis phytohormones to improve plant performance and stress tolerance. In another study, *Bacillus* sp. WM13-24 and *Pseudomonas* sp. M30-35 increased the drought tolerance of ryegrass through improving the activities of antioxidant enzymes and regulating ABA signaling [14]. *Bacillus* spp., one of the most frequently used PGPR, exhibits bio-control and bio-fertilizer traits, with a growing commercial market worldwide. *Bacillus amyloliquefaciens* QST713 (formerly *subtilis*), as a broad-spectrum bio-fungicide, has been applied in fruit and vegetable production [15,16]. In addition, it has proven effective in increasing Fe and P uptake by plants [17,18] and improving growth and development of the root system to allow greater absorption of water and nutrients [19,20]. In addition, it is capable of secreting IAA and EPS, producing of siderophores and forming biofilms to protect plants from adverse environmental conditions [21,22].

Alfalfa (*Medicago sativa* L.) is an excellent perennial forage crop, with high biomass yield and good feed quality [23]. However, drought stress largely inhibits the productivity and quality of alfalfa [10,24]. Previous studies have reported that biomass and photosynthesis of alfalfa significantly decrease, by 49%, due to water stress [10]. Therefore, enhancing the drought tolerance of alfalfa is essential for maintaining and increasing its greater economic value. The present study was executed to evaluate the effects of QST713 on plant growth, photosynthesis and antioxidant defense, to improve the drought resistance of cultivated alfalfa.

## 2. Materials and Methods

### *2.1. The Source of Bacteria*

*B. amyloliquefaciens* QST713, isolated from the commercial formulation of Zhuorun®, Bayer CropScience, originated from North America. It was grown on Luria-Bertani (LB) medium for 12 h at 28 °C with 180 rpm, then inoculated in 100 mL of an LB medium at 2% inoculum and cultured for 24 h. Cells were collected by centrifugation (5000 rpm for 10 min, 4 °C) and resuspended in distilled water to generate $1.0 \times 10^8$ colony forming units (CFU)/mL for inoculation, which was verified by standard plate counts on LB agar medium.

### *2.2. Plant Materials and Treatments*

The experiment was carried out in an environment-controlled artificial intelligence climate chamber under LED light (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity) with a photoperiod of 14 h at 28/18 °C (day/night) and a relative humidity of 60% at Shanxi Agricultural University, China. Two alfalfa cultivars, namely 'Galalxie Max' and 'Saidi 7', referred to as 'drought-tolerant' and 'drought-sensitive', respectively, were used as test materials and were obtained from the Pratacultural Science Group of Shanxi Agricultural University. The seeds with uniform size were sown in plastic pots ($7 \times 7 \times 7$ cm) containing equal amounts of sterilized coconut bran (substrate); each plastic pot contained eight seedlings. The maximum water-holding capacity of the substrate was determined before the test. Daily weighing was performed for quantitative watering, so that the substrate moisture

content of each pot was consistent. After two weeks, 20 mL of Hoagland nutrient solution was irrigated every 2 d to ensure the nutrients needed for the normal growth of seedlings.

When the seedlings reached 2 months old, they were divided into 4 treatments: (1) CK, untreated plants (control): the substrate moisture was maintained at 75–80% of the maximum substrate water holding capacity; (2) CK + QST713: plants were inoculated with 5 mL of QST713 at 1, 11 and 21 days of the experiment; (3) MD: plants were challenged under drought stress with 55–60% of maximum substrate water holding capacity; (4) MD + QST713: plants were inoculated with 5 mL of QST713 at 1, 11 and 21 days after drought stress. The whole experiment was carried out for 30 days. To investigate the dynamic changes of different varieties of alfalfa in response to QST713 under drought stress, samples were measured over long-term (10, 20 and 30 d) treatment. The experiments were randomized, with three replicates containing 96 plants per treatment.

### *2.3. Assessment of Dry and Fresh Weight*

The fresh weight of stems and roots was directly weighed using an electronic scale. Samples were dried in an oven at 105 °C for 15 min and achieved constant weight at 80 °C.

### *2.4. Determination of Leaf Relative Water Content (RWC)*

A total of 10 expanded leaves from the same height of plant were selected and weighed immediately ($W_1$) and then immersed in distilled water for 6 h at 4 °C in the dark, following being weighed to determine their turgor weight ($W_2$). Then, samples were dried in oven at 80 °C for 24 h, and their dry weight ($W_3$) was recorded. The formula for calculating RWC was as follows:

$$\text{RWC (\%)} = [(W_1 - W_3)/(W_2 - W_3)] \times 100\% \quad (1)$$

### *2.5. Measurement of Gas-Exchange Parameters, Carotenoids and Chlorophyll Contents*

The net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and intercellular $CO_2$ concentration (Ci) were measured using a LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) on a fully expanded leaf.

Leaf chlorophyll and carotenoid contents were measured following the method of Lichtenthaler and Wellburm [25]. In brief, 0.05 g of fresh leaf tissue was mixed with 15 mL of 95% ethanol and transferred to a dark environment. After the green fading, spectrophotometric analysis was performed at 665 nm for chlorophyll a (Chl a), 649 nm for chlorophyll b (Chl b) and 470 nm for carotenoids.

### *2.6. Superoxide Anion ($O_2.^-$) Generation Rate and Hydrogen Peroxide ($H_2O_2$)*

The rate of superoxide anion ($O_2.^-$) generation was determined by the hydroxylamine reaction method [26]. $H_2O_2$ contents were determined using a reagent kit (Solarbio® Life Sciences Co., Ltd., Beijing, China).

### *2.7. Assays of Antioxidant Enzyme*

Superoxide dismutase (SOD) was examined by the nitro blue tetrazolium (NBT) method of Giannopolitis and Ries [27]. The inhibition of 50% NBT is defined as one SOD unit. Catalase (CAT) activity was assayed following the method of Chance and Maehley [28]. Peroxidase (POD) activity was evaluated following the method of Kochba et al. [29]. The method of Nakano and Asada [30] was used to determine ascorbate peroxidase (APX) activity.

### *2.8. Statistical Analysis*

All data were examined by SPSS 20.0 program (SPSS Inc., Chicago, IL, USA) using an LSD test ($p < 0.05$), and graphs were constructed in Design Expert 8.0 software.

## 3. Results

### *3.1. The Effects of Strain QST713 on the Biomass of Alfalfa Seedlings under Drought Stress*

The drought stress in the present study resulted in a significant reduction in aboveground biomass (shoot fresh and dry weigh) and underground biomass (root fresh and dry weigh) in both cultivars compared to CK (Table 1). However, the plants with QST713 inoculation showed higher biomass as compared to plants without QST713 under normal and stressed plant conditions in both cultivars. In particular, under drought stress, the plants with QST713 increased shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of 'Galalxie Max' by 12.07–61.94%, 8.09–53.33%, 18.18–57.97% and 14.87–100.00%, respectively, and 'Saidi 7' by 21.20–56.72%, 25.78–59.57%, 32.02–111.54% and 28.57–118.18%, respectively, than those in plants without QST713 application.

**Table 1.** Effect of drought stress and QST713 application on the biomass of alfalfa seedlings in two cultivars.

| Treated Days | Varieties | Treatments | Shoot Fresh Weight (g) | Shoot Dry Weight (g) | Root Fresh Weight (g) | Root Dry Weight (g) |
|---|---|---|---|---|---|---|
| 10 | Galalxie Max | CK | 3.24 ± 0.20 b | 0.48 ± 0.04 b | 1.10 ± 0.01 b | 0.12 ± 0.01 b |
| | | CK + QST713 | 4.25 ± 0.34 a | 0.61 ± 0.031 a | 1.43 ± 0.07 a | 0.17 ± 0.02 a |
| | | MD | 1.34 ± 0.021 d | 0.20 ± 0.02 d | 0.69 ± 0.03 c | 0.06 ± 0.00 c |
| | | MD + QST713 | 2.17 ± 0.02 c | 0.29 ± 0.021 c | 1.09 ± 0.19 b | 0.12 ± 0.01 b |
| | Saidi 7 | CK | 1.80 ± 0.06 b | 0.22 ± 0.00 b | 0.74 ± 0.07 b | 0.05 ± 0.00 b |
| | | CK + QST713 | 2.27 ± 0.15 a | 0.34 ± 0.03 a | 1.22 ± 0.16 a | 0.10 ± 0.01 a |
| | | MD | 0.67 ± 0.02 d | 0.10 ± 0.00 d | 0.26 ± 0.01 d | 0.03 ± 0.00 c |
| | | MD + QST713 | 1.05 ± 0.09 c | 0.15 ± 0.01 c | 0.55 ± 0.03 c | 0.05 ± 0.00 b |
| 20 | Galalxie Max | CK | 9.34 ± 0.2 b | 1.40 ± 0.12 b | 2.84 ± 0.05 b | 0.43 ± 0.02 b |
| | | CK + QST713 | 11.57 ± 0.24 a | 1.84 ± 0.15 a | 3.86 ± 0.11 a | 0.57 ± 0.03 a |
| | | MD | 4.82 ± 0.80 d | 0.75 ± 0.11 c | 1.67 ± 0.04 d | 0.17 ± 0.01 d |
| | | MD + QST713 | 7.00 ± 0.33 c | 1.15 ± 0.06 b | 2.20 ± 0.07 c | 0.28 ± 0.02 c |
| | Saidi 7 | CK | 9.22 ± 0.16 b | 1.47 ± 0.07 b | 2.36 ± 0.19 b | 0.28 ± 0.01 b |
| | | CK + QST713 | 11.84 ± 0.68 a | 2.69 ± 0.12 a | 4.27 ± 0.26 a | 0.95 ± 0.07 a |
| | | MD | 3.38 ± 0.08 d | 0.47 ± 0.00 d | 1.13 ± 0.01 d | 0.11 ± 0.00 c |
| | | MD + QST713 | 5.18 ± 0.04 c | 0.75 ± 0.04 c | 1.75 ± 0.00 c | 0.24 ± 0.01 b |
| 30 | Galalxie Max | CK | 12.97 ± 0.12 b | 2.98 ± 0.06 a | 3.66 ± 0.26 a | 1.00 ± 0.04 b |
| | | CK + QST713 | 13.76 ± 0.05 a | 3.19 ± 0.14 a | 3.97 ± 0.33 a | 1.24 ± 0.02 a |
| | | MD | 7.62 ± 0.09 d | 1.73 ± 0.01 b | 1.76 ± 0.05 b | 0.47 ± 0.03 c |
| | | MD + QST713 | 8.54 ± 0.11 c | 1.87 ± 0.03 b | 2.08 ± 0.06 b | 0.54 ± 0.03 c |
| | Saidi 7 | CK | 10.37 ± 0.41 b | 2.26 ± 0.09 b | 3.49 ± 0.14 b | 0.85 ± 0.01 b |
| | | CK + QST713 | 12.51 ± 0.07 a | 2.68 ± 0.12 a | 4.27 ± 0.10 a | 1.08 ± 0.06 a |
| | | MD | 5.66 ± 0.10 d | 1.28 ± 0.05 d | 1.78 ± 0.01 d | 0.42 ± 0.01 d |
| | | MD + QST713 | 6.86 ± 0.02 c | 1.61 ± 0.05 c | 2.35 ± 0.04 c | 0.54 ± 0.00 c |

Data are the mean ± SE ($n$ = 3). Different letters indicate significant differences from each other ($p < 0.05$).

### *3.2. The Effects of Strain QST713 on the Leaf Relative Water Content of Alfalfa Seedlings under Drought Stress*

The drought stress remarkably decreased the leaf relative water content in both cultivars compared to CK (Figure 1). However, the plants with QST713 inoculation showed higher RWC as compared to plants without QST713 under normal and stressed plant conditions in both cultivars. With the drought time, the RWC showed significant increase in the cultivars of 'Galalxie Max' and 'Saidi 7' after inoculation with QST713: 10.29–15.05% and 8.91–14.47%, respectively (compared with the MD treatment).

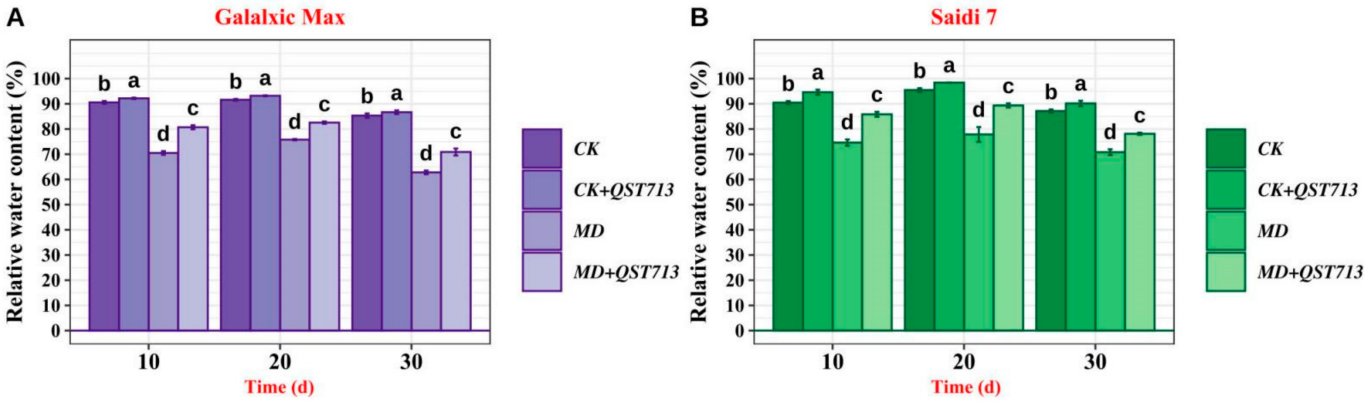


**Figure 1.** Effect of drought stress and QST713 application on the leaf relative water content (RWC) of alfalfa seedlings in two cultivars ((**A**) Galalxic Max; (**B**) Saidi 7). Data are the mean ± SE ($n$ = 3). Different letters indicate significant differences from each other ($p < 0.05$).

### *3.3. The Effects of Strain QST713 on Photosynthetic Gas Exchange Parameters of Alfalfa Seedlings under Drought Stress*

The drought stress significantly reduced Pn, Tr, Gs and Ci in both cultivars compared to CK (Figure 2). However, under well-watered conditions, inoculation with QST713 significantly improved Pn and Tr of 'Galalxie Max' by 1.16- and 1.21-fold, respectively, and 'Saidi 7' by 1.24- and 1.15-fold, respectively, compared to CK on the 20th day. Under drought stress, the plants with QST713 had increased Pn, Tr, Gs and Ci of 'Galalxie Max' by 1.11–11.32%, 13.04–31.00%, 7.14–100.00% and 1.89–2.38%, respectively, and 'Saidi 7' by 9.59–30.98%, 4.23–49.28%, 2.00–50.00% and 0.71–7.71%, respectively, than those in plants without QST713 application.

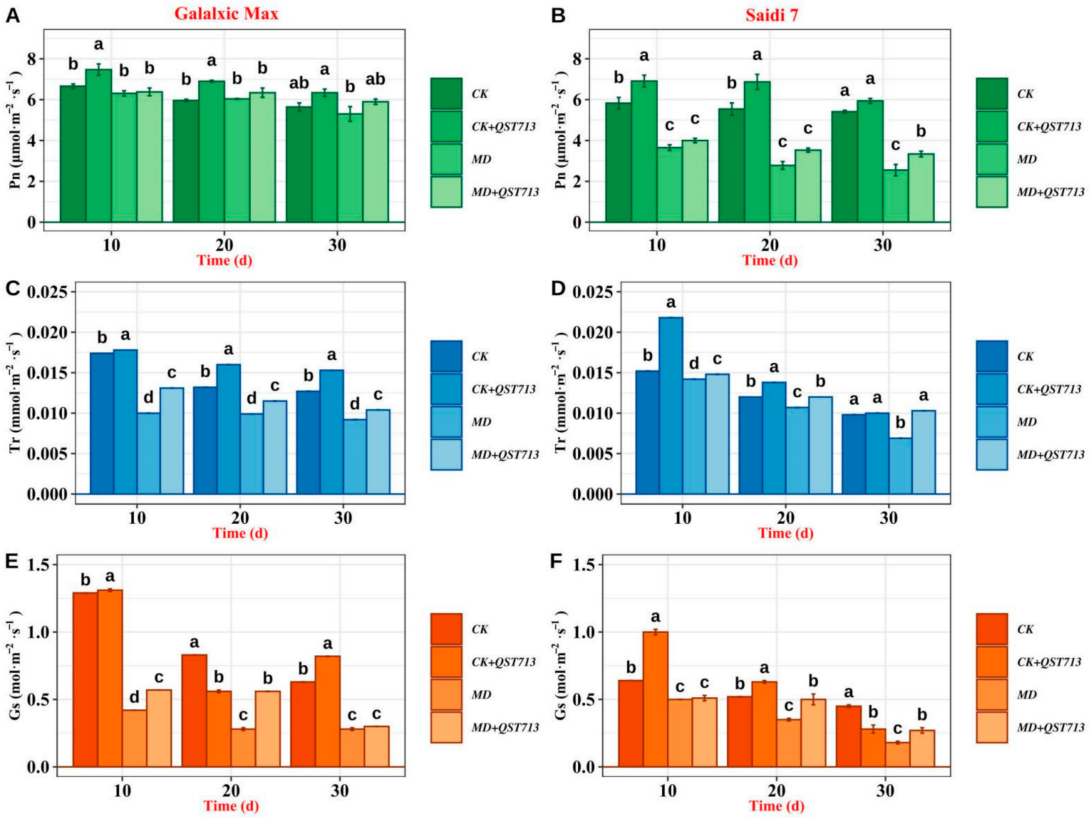


**Figure 2.** *Cont.*

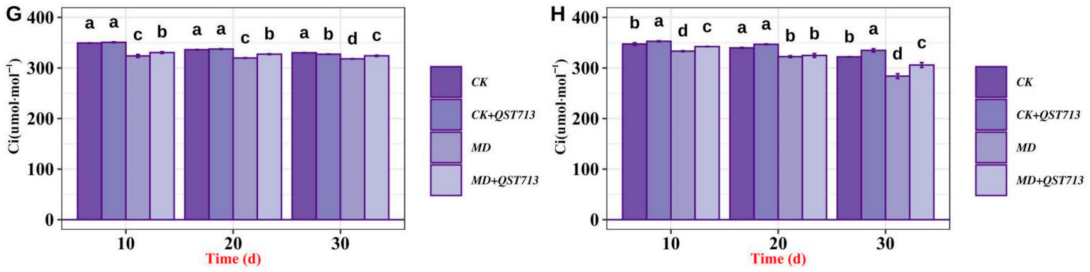


**Figure 2.** Effect of drought stress and QST713 application on photosynthetic rate (Pn; (**A**,**B**)), transpiration rate (Tr; (**C**,**D**)),stomatal conductance (Gs; (**E**,**F**)) and intercellular $CO_2$ (Ci; (**G**,**H**)) of alfalfa seedlings in two cultivars ((**A**,**C**,**E**,**G**), Galalxic Max; (**B**,**D**,**F**,**H**), Saidi 7). Data are the mean ± SE ($n$ = 3). Different letters indicate significant differences from each other ($p < 0.05$).

### *3.4. The Effects of Strain QST713 on Photosynthetic Pigments of Alfalfa Seedlings under Drought Stress*

Drought stress markedly decreased the content of Chl a, Chl b and carotenoids in the leaves of alfalfa of both cultivars as compared to CK (Figure 3). However, the plants with bacterial inoculation showed higher contents of Chl a, Chl b and carotenoids as compared to non-inoculated plants under drought conditions. QST713 significantly increased Chl a of 'Galalxie Max' by 6.85–15.43% and 'Saidi 7' by 11.48–15.57% compared with the non-inoculated treatment under stressed conditions.

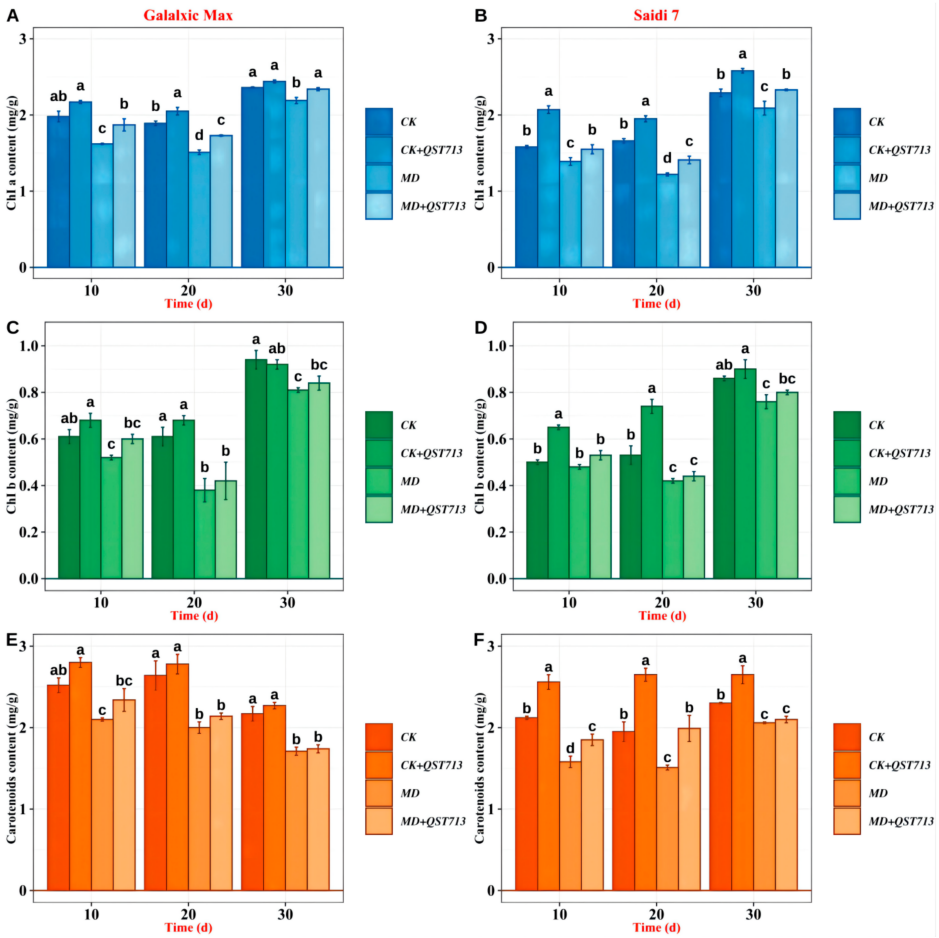


**Figure 3.** Effect of drought stress and QST713 application on the content of Chl a (**A**,**B**), Chl b (**C**,**D**) and carotenoids (**E**,**F**) of alfalfa seedlings in two cultivars ((**A**,**C**,**E**), Galalxic Max; (**B**,**D**,**F**), Saidi 7). Data are the mean ± SE ($n$ = 3). Different letters indicate significant differences from each other ($p < 0.05$).

### *3.5. The Effects of Strain QST713 on Reactive Oxygen Content of Alfalfa Seedlings under Drought Stress*

As shown in Figure 4, compared to CK, the content of $O_2.^-$ and $H_2O_2$ increased in the leaves of alfalfa of both cultivars under drought conditions and were decreased in the treatment with QST713. On the 30th day, inoculation with QST713 decreased the content of $O_2.^-$ and $H_2O_2$ of 'Da Yinhe' by 59.65% and 43.82%, respectively, and 'Saidi 7' by 29.49% and 10.96%, respectively, compared to non-inoculated plants under stress conditions.

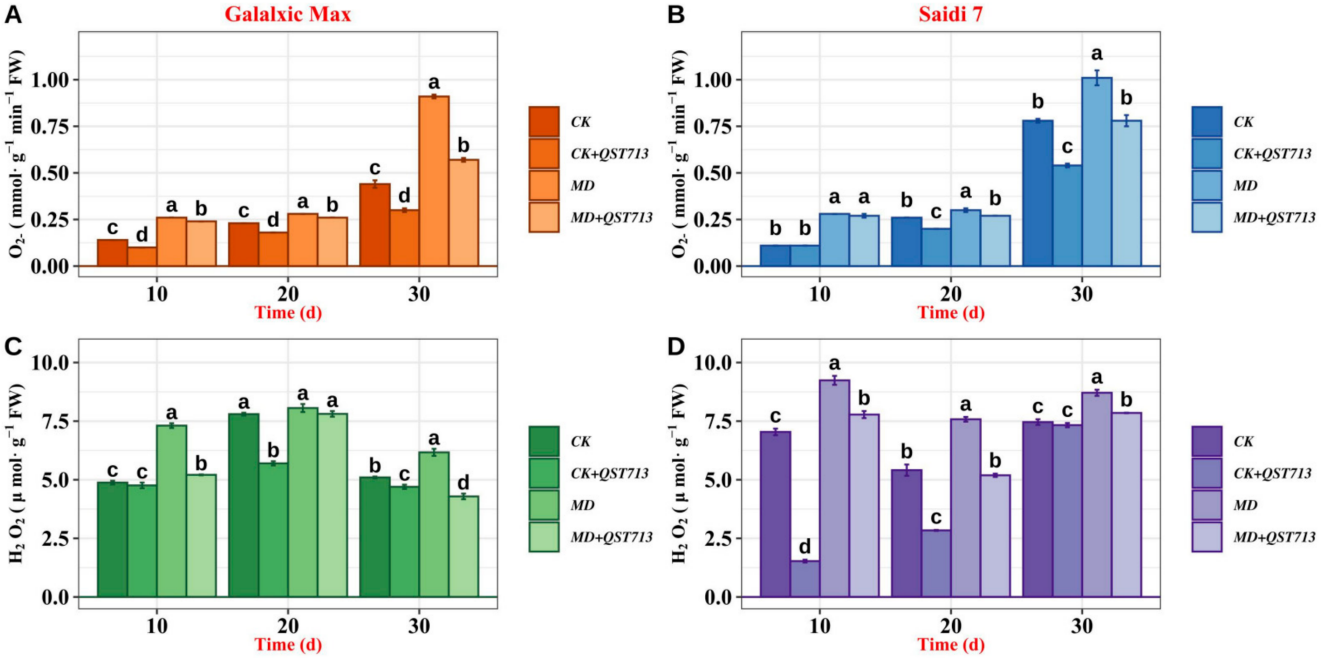


**Figure 4.** Effect of drought stress and QST713 application on the content of $O_2.^-$ (**A**,**B**) and $H_2O_2$ (**C**,**D**) of alfalfa seedlings in two cultivars ((**A**,**C**), Galalxic Max; (**B**,**D**), Saidi 7). Data are the mean ± SE ($n$ = 3). Different letters indicate significant differences from each other ($p < 0.05$).

### *3.6. The Effects of Strain QST713 on Antioxidant Enzyme Activity of Alfalfa Seedlings under Drought Stress*

QST713 inoculation remarkably increased the activities of SOD, POD, CAT and APX in both cultivars leaves under drought stress conditions (Figure 5). However, with the drought time, the four enzyme activities showed greater differences in the cultivars of 'Galalxie Max' and 'Saidi 7' after inoculation with QST713; the multiples were 1.05–1.53 times and 1.04–1.16 times, respectively (compared with the MD).

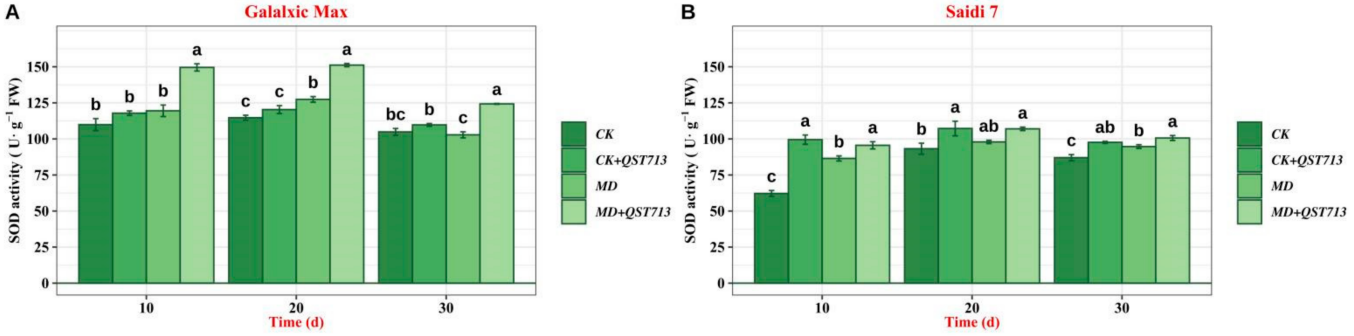


**Figure 5.** *Cont.*

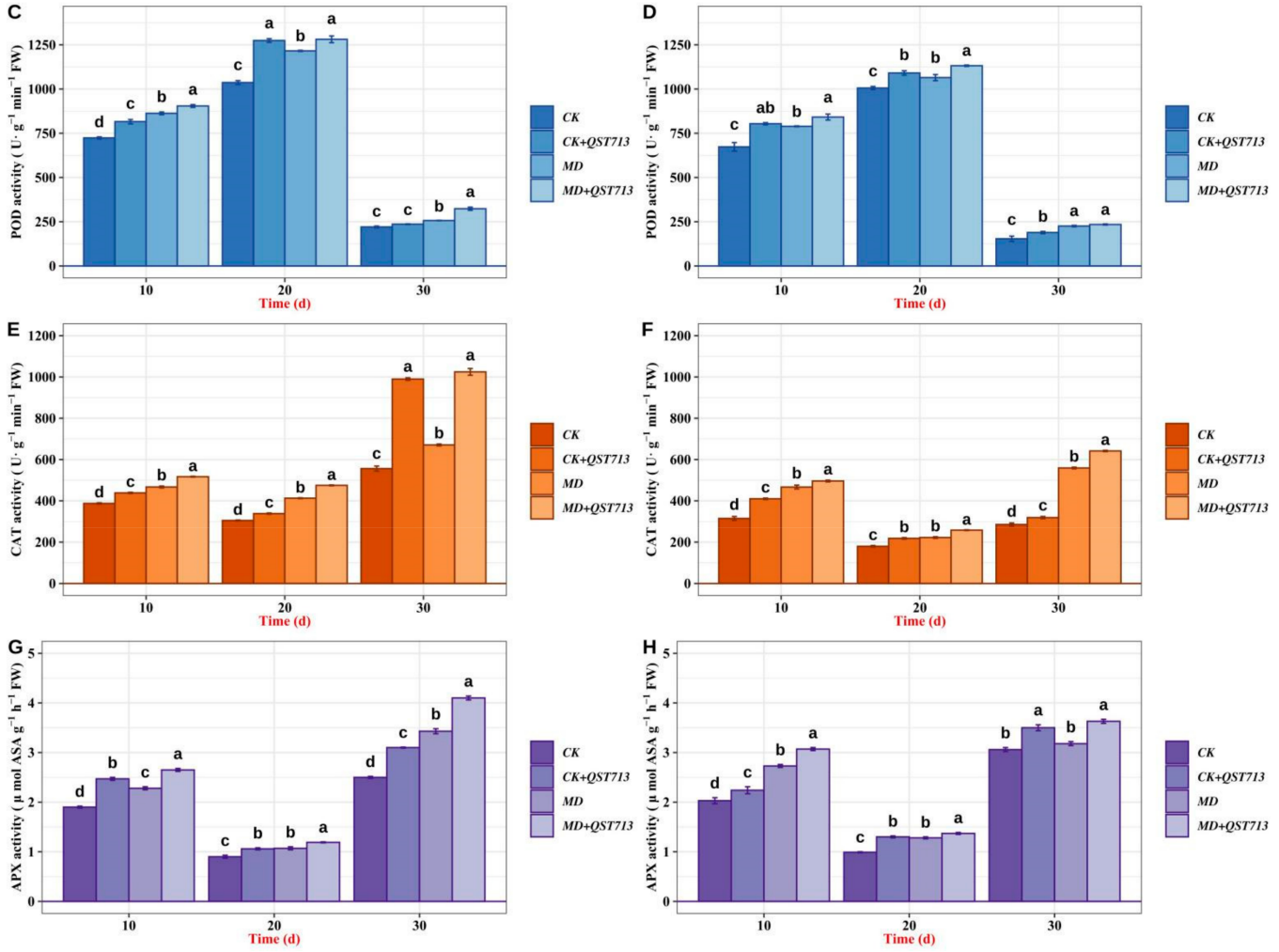


**Figure 5.** Effect of drought stress and QST713 application on antioxidative enzyme activities of SOD (**A**,**B**), POD (**C**,**D**), CAT (**E**,**F**) and APX (**G**,**H**) in leaves of alfalfa seedlings in two cultivars ((**A**,**C**,**E**,**G**), Galalxic Max; (**B**,**D**,**F**,**H**), Saidi 7). Data are the mean ± SE ($n$ = 3). Different letters indicate significant differences from each other ($p < 0.05$).

## 4. Discussion

Drought, causing morphological, physiological and biochemical changes in plants, negatively affects plant growth and productivity [9]. PGPR can ameliorate the performance of plants challenged with environment stresses such as drought, salinity and heat stress by enhancing their growth, nutrient and water uptake, photosynthesis and antioxidant systems [3,31]. In this study, plant biomass was significantly reduced under drought stress (Table 1). However, QST713 alleviated the deleterious effects of water stress and increased biomass content in both alfalfa cultivars ('tolerant' and 'sensitive' to drought). These results indicated that drought stress inhibited growth in alfalfa seedlings and that QST713 application can relieve at least a portion of the stress and promote plant growth. Other reports demonstrated that PGPR enhanced the plant biomass (fresh and dry weight of roots and shoots) under drought stress [12,14]. PGPR can likely effectively enhance the growth of plant root and shoot systems by balancing the levels of plant hormones like GAs, cytokinins, auxins and ABA, aiding the plant in surviving drought stress [11].

Higher RWC can prevent the reactive oxygen species and osmotic stresses in plants caused by droughts and potentially contribute to greater plant production [32,33]. In the present study, the plants with QST713 inoculation significantly increased RWC in both cultivars over plants without QST713 under drought stress (Figure 1). This indicates the application of QST713 can effectively alleviate the damage of alfalfa leaves caused by

drought stress. Previous studies have also reported enhancement of the relative water content by PGPR [12,34]. This may suggest that PGPR has the potential to regulate the water content by altering the hydraulic conductivity and stomatal openings [35]. Specifically, PGPR increased the $K^+$ content of plants under stress conditions, which is responsible for stomata movements in response to changes in leaf water status [36,37].

It is well-known that leaf gas exchange parameters and chlorophyll content can be examined to analyze the tolerance of drought stress [38,39]. A reduction in gas exchange parameters is related to the set of metabolic responses triggered by water stress, such as a reduction in Ci due to the lower Gs, and the resulting possible damage to the photosynthetic apparatus [40]. In the current study, the adverse effect of drought stress on Pn, Tr, Gs and Ci was obvious in both cultivars (Figure 2). However, inoculation with QST713 improved these parameters in stress treatment in comparison to the non-inoculated seedlings, indicating that QST713 enhanced the photosynthetic capacity of alfalfa leaves and alleviated the effect of drought stress. Azizi et al. [41] reported that myrtle plants with the inoculation of PGPR showed an increase in the rate of Pn, Tr and Gs under drought stress. Thus, by increasing water absorption, PGPR delay stomatal closure and transpiration under water stress and positively influence photosynthetic apparatus activity, resulting in a smaller decline in photosynthesis.

In addition, substantial research has documented that drought stress significantly reduces the content of plant photosynthetic pigments (chlorophyll and carotenoids) [42,43]. Chlorophyll a and chlorophyll b, as the main photosynthetic pigments of plants, will directly affect the progress of photosynthesis. Carotenoids are related to the dissipation of excess excitation light energy by plants, which can enhance the resistance of plant photosynthetic organs to strong light. In this study, drought stress significantly decreased the content of Chl a, Chl b and carotenoids (Figure 3). However, inoculation with QST713 improved these parameters in stress treatment in comparison to the non-inoculated seedlings. Li et al. [44] reported that PGPR prevented chlorophyll degradation in stressed plants by improving their water relations and increasing RWC, as the shortage of water in leaves leads to decreased biosynthesis of chlorophyll and promotes the decomposition of chlorophyll. Therefore, these results in combination with other reports indicated that PGPR are beneficial to maintain the normal photosynthetic capacity of alfalfa seedlings and improve the adaptability of alfalfa plants to drought stress.

Severe oxidation damage was found in leaf chloroplasts by increased $O_2.^-$ and $H_2O_2$ content under abiotic stress. Substantial research has shown that the antioxidant enzymes (SOD, POD, CAT and APX) perform considerable functions in ROS obliteration and hydroxyl radical reduction. SOD converts $O_2.^-$ to $H_2O_2$, which is then reduced to $H_2O$ and $O_2$ either by APX or by CAT in cytoplasm and other cell components [45]. POD plays an important role in catalyzing $H_2O_2$ to $H_2O$ and $O_2$ [46]. In this experiment, the antioxidant enzyme activities in plants with PGPR were significantly higher than those without PGPR under drought stress (Figure 5). In addition, PGPR could effectively prevent the accumulation of $O_2.^-$ and $H_2O_2$ in plant cells caused by drought stress (Figure 4). This indicated that QST713 alleviated the drought stress effects in alfalfa seedlings by the induction of antioxidant enzymes. This is similar to previous reports that *B. pumilus* inoculation improved the activity of CAT, resulting in decreased $H_2O_2$ accumulation and the production rate of $O_2.^-$ induced by drought stress [3]. This may be that PGPR produce various bacterial molecules such as flagellar proteins, chitin and lipopeptide surfactants, which promote changes in host physiology, leading to an overexpression of plant defensive chemicals, including antioxidative enzymes, chitinases and phenylalanine ammonia-lyase [47].

## 5. Conclusions

This investigation clearly demonstrated that QST713 can mitigate the injury to alfalfa seedlings caused by drought stress through enhancing the activity of antioxidant enzymes and ameliorating photosynthetic performance. Therefore, QST713 could be considered as a promising biofertilizer in arid areas. However, further studies using field experiments to

identify and validate the effectiveness of this biofertilizer in drought-stressed affected areas for practical applications are needed.

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## References

1. Kour, D.; Rana, K.L.; Sheikh, I.; Kumar, V.; Yadav, A.N.; Dhaliwal, H.S.; Saxena, A.K. Alleviation of drought stress and plant growth promotion by *Pseudomonas libanensis* EU-LWNA-33, a drought-adaptive phosphorus-solubilizing bacterium. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.* **2019**, *90*, 785–795. [CrossRef]
2. Azib, S.; Cheloufi, H.; Attab, S.; Bouras, N. Improvement of alfalfa growth under water stress by inoculation with Sinorhizobium meliloti strains from the Algerian Sahara. *Int. J. Sci. Res.* **2019**, *75*, 35–43. [CrossRef]
3. Khalilpour, M.; Mozafari, V.; Abbaszadeh-Dahaji, P. Tolerance to salinity and drought stresses in pistachio (*Pistacia vera* L.) seedlings inoculated with indigenous stress-tolerant PGPR isolates. *Sci. Hortic.* **2021**, *289*, 110440. [CrossRef]
4. Laxa, M.; Liebthal, M.; Telman, W.; Chibani, K.; Dietz, K.J. The role of the plant antioxidant system in drought tolerance. *Antioxidants* **2019**, *8*, 94. [CrossRef] [PubMed]
5. Shi, L.; Du, N.S.; Yuan, Y.H.; Shu, S.; Sun, J.; Guo, S.R. Vinegar residue compost as a growth substrate enhances cucumber resistance against the Fusarium wilt pathogen *Fusarium oxysporum* by regulating physiological and biochemical responses. *Environ. Sci. Pollut. R* **2016**, *23*, 18277–18287. [CrossRef]
6. Lulsdorf, M.M.; Hy, Y.; Smh, S.; Vandenberg, A.; Abrams, S.R. Endogenous hormone profiles during early seed development of *C. arietinum* and *C. anatolicum*. *Plant Growth Regul.* **2013**, *71*, 191–198. [CrossRef]
7. Batool, T.; Ali, S.; Seleiman, M.F.; Naveed, N.H.; Ali, A.; Ahmed, K.; Abid, M.; Rizwan, M.; Shahid, M.R.; Alotaibi, M.; et al. Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. *Sci. Rep.* **2020**, *10*, 16975. [CrossRef]
8. Moreno-Lora, A.; Garrido, R.R.; Delgado, A. *Bacillus subtilis* QST713 and cellulose amendment enhance phosphorus uptake while improving zinc biofortification in wheat. *Appl. Soil Ecol.* **2019**, *142*, 81–89. [CrossRef]
9. Chandra, P.; Wunnava, A.; Verma, P.; Chandra, A.; Sharma, R.K. Strategies to mitigate the adverse effect of drought stress on crop plants—Influences of soil bacteria: A review. *Pedosphere* **2021**, *31*, 496–509. [CrossRef]
10. Kang, S.M.; Khan, M.A.; Hamayun, M.; Kim, L.R.; Kwon, E.H.; Kang, Y.S.; Kim, K.Y.; Park, J.J.; Lee, I.J. Phosphate-solubilizing *Enterobacter ludwigii* AFFR02 and *Bacillus megaterium* Mj1212 rescues alfalfa's growth under post-drought stress. *Agriculture* **2021**, *11*, 485. [CrossRef]
11. Ahluwalia, O.; Singh, P.C.; Bhatia, R. A review on drought stress in plants: Implications, mitigation and the role of plant growth promoting rhizobacteria. *Resour. Environ. Sustain.* **2021**, *5*, 100032. [CrossRef]
12. Ansari, F.A.; Jabeen, M.; Ahmad, I. *Pseudomonas azotoformans* FAP5, a novel biofilm-forming PGPR strain, alleviates drought stress in wheat plant. *Int. J. Environ. Sci. Tech.* **2021**, *18*, 3855–3870. [CrossRef]
13. Mariotti, L.; Scartazza, A.; Curadi, M.; Picciarelli, P.; Toffanin, A. *Azospirillum baldaniorum* Sp245 induces physiological responses to alleviate the adverse effects of drought stress in purple basil. *Plants* **2021**, *10*, 1141. [CrossRef] [PubMed]
14. He, A.; Niu, S.Q.; Yang, D.; Ren, W.; Zhao, L.Y.; Sun, Y.Y.; Meng, L.S.; Zhao, Q.; Paré, P.W.; Zhang, J.L. Two PGPR strains from the rhizosphere of Haloxylon ammodendron promoted growth and enhanced drought tolerance of ryegrass. *Plant Physiol. Biochem.* **2021**, *161*, 74–85. [CrossRef] [PubMed]
15. Sabo, R.; Kopáková, A.; Hamarová, L.; Maruáková, C.I.; Mudroňová, D.; Sabová, L.; Javorsky, P.; Legáth, J. Sublethal effects of commercial plant protection product containing spores *Bacillus amyloliquefaciens* QST 713 (formerly *subtilis*) on winter adult honeybees. *Apidologie* **2020**, *51*, 226–239. [CrossRef]
16. Antje, R.; Lise, N.J. Biological control of yellow rust of wheat (*Puccinia striiformis*) with Serenade®ASO (*Bacillus subtilis* strain QST713). *Crop Prot.* **2017**, *93*, 1–8. [CrossRef]
17. García-López, A.M.; Avilés, M.; Delgado, A. Effect of various microorganisms on phosphorus uptake from insoluble Ca-phosphates by cucumber plants. *J. Plant Nutr. Soil Sci.* **2016**, *179*, 454–465. [CrossRef]

18. García-López, A.M.; Recena, R.; Aviles, M.; Delgado, A. Effect of *Bacillus subtilis* QST713 and *Trichoderma asperellum* T34 on P uptake by wheat and how it is modulated by soil properties. *J. Soils Sediments* **2018**, *18*, 727–738. [CrossRef]
19. Altunta, O. A comparative study on the effects of different conventional, organic and bio-fertilizers on broccoli yield and quality. *Appl. Ecol. Environ. Res.* **2018**, *16*, 1595–1608. [CrossRef]
20. Moretti, L.G.; Crusciol, C.A.C.; Kuramae, E.E.; Bossolani, J.W.; Moreira, A.; Costa, N.R.; Alves, C.J.; Pascoaloto, I.M. Effects of growth-promoting bacteria on soybean root activity, plant development, and yield. *Agron. J.* **2020**, *112*, 418–428. [CrossRef]
21. García-López, A.M.; Delgado, A. Effect of Bacillus subtilis on phosphorus uptake by cucumber as affected by iron oxides and the solubility of the phosphorus source. *Agric. Food Sci.* **2016**, *25*, 216–224. [CrossRef]
22. Pandin, C.; Darsonval, M.; Mayeur, C.; Coq, D.L.; Aymerich, S.; Briandet, R. Biofilm formation and synthesis of antimicrobial compounds by the biocontrol agent *Bacillus velezensis* QST713 in *Agaricus bisporus* compost micromodel. *Appl. Environ. Microbiol.* **2019**, *85*, e00327-19. [CrossRef] [PubMed]
23. Baha, N. Impact of bio-fertilizers on germination and early seedling growth of Alfalfa (*Medicago sativa* L.) under salt stress. *Acta Physiol. Plant* **2021**, *43*, 81. [CrossRef]
24. Li, Z.H.; Zou, Y.; Li, Y.P.; Sun, C.C.; Liu, Y.; Cui, L.W.; Liu, J.; Yang, Z.M.; Chen, Y. Exploring the salt- and drought-tolerant genes of alfalfa through expression library screening strategy. *Grass Res.* **2021**, *1*, 11. [CrossRef]
25. Lichtenthaler, K.H.; Wellburm, A.R. Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *603*, 591–593. [CrossRef]
26. Elstner, E.F.; Heupel, A. Inhibition of nitrite formation from hydroxylammoniumchloride: A simple assay for superoxide dismutase. *Anal. Biochem.* **1976**, *70*, 616–620. [CrossRef]
27. Giannopolitis, C.N.; Ries, S.K. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* **1977**, *59*, 309–314. [CrossRef]
28. Chance, B.; Maehley, A. Assay of catalase and peroxidase. *Method Enzymol.* **1955**, *2*, 764–775.
29. Kochba, J.; Lavee, S.; Spiegel-Roy, P. Differences in peroxidase activity and isoenzymes in embryogenic and non-embryogenic 'Shamouti' orange ovular callus lines. *Plant Cell Physiol.* **1977**, *18*, 463–467. [CrossRef]
30. Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged byascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **1981**, *22*, 867–880. [CrossRef]
31. Gupta, S.; Pandey, S. ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. *Front Microbiol.* **2019**, *10*, 1506–1522. [CrossRef]
32. Ngumbi, E.; Kloepper, J. Bacterial-mediated drought tolerance: Current and future prospects. *Appl. Soil Ecol.* **2016**, *105*, 109–125. [CrossRef]
33. García, J.E.; Maroniche, G.; Creus, C.; Suárez-Rodríguez, R.; Ramirez-Trujillo, J.A.; Groppa, M. In vitro PGPR properties and osmotic tolerance of different Azospirillum native strains and their effects on growth of maize under drought stress. *Microbiol. Res.* **2017**, *202*, 21–29. [CrossRef] [PubMed]
34. Kazeminasab, A.; Yarnia, M.; Lebaschy, M.H.; Mirshekari, B.; Rejali, F. The effect of vermicompost and PGPR on physiological traits of lemon balm (*Melissa officinalis* L.) plant under drought stress. *J. Med. Plants By-Prod.* **2016**, *2*, 135–144. [CrossRef]
35. Zheng, W.J.; Zeng, S.Q.; Bais, H.; Lamanna, J.M.; Hussey, D.S.; Jacobson, D.L.; Jin, Y. Plant growth-promoting rhizobacteria (PGPR) reduce evaporation and increase soil water retention. *Water Resour. Res.* **2018**, *54*, 3673–3687. [CrossRef]
36. Abd El-Ghany, T.M.; Masrahi, Y.S.; Mohamed, A.; Abboud, A.; Alawlaqi, M.M.; Elhussieny, N.I. Maize (*Zea Mays* L.) growth and metabolic dynamics with plant growth promoting rhizobacteria under salt stress. *J. Plant Pathol. Microb.* **2015**, *6*, 1000305. [CrossRef]
37. Kang, S.M.; Khan, A.L.; Waqas, M.; You, Y.H.; Kim, J.H.; Kim, J.G.; Hamayun, M.; Lee, I.J. Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. *J. Plant Interact.* **2014**, *9*, 673–682. [CrossRef]
38. Bürling, K.; Cerovic, Z.G.; Cornic, G.; Ducruet, J.M.; Noga, G.; Hunsche, M. Fluorescence-based sensing of drought-induced stress in the vegetative phase of four contrasting wheat genotypes. *Environ. Exp. Bot.* **2013**, *89*, 51–59. [CrossRef]
39. Mishra, S.K.; Khan, M.H.; Misra, S.; Dixit, V.K.; Gupta, S.; Tiwari, S.; Gupta, S.C.; Chauhan, P.S. Drought tolerant *Ochrobactrum* sp. inoculation performs multiple roles in maintaining the homeostasis in *Zea mays* L. subjected to deficit water stress. *Plant Physiol. Bioch.* **2020**, *150*, 1–14. [CrossRef]
40. Nunes, T.A.; Fernandes, H.M.; Santos, R.S.; Kikuchi, C.A.; Martinez, D.; Antonio, P.J.; Caixeta, O.H.; Edmilson, B.; Renta, S.M. Enhanced drought tolerance in seedlings of neotropical tree species inoculated with plant growth-promoting bacteria. *Plant Physiol. Bioch.* **2018**, *130*, 277–288. [CrossRef]
41. Azizi, S.; Kouchaksaraei, M.T.; Hadian, J.; Abad, A.R.F.N.; Sanavi, S.A.M.M.; Ammer, C.; Bader, M.K.F. Dual inoculations of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria boost drought resistance and essential oil yield of common myrtle. *For. Ecol. Manag.* **2021**, *497*, 119478. [CrossRef]
42. Akbari, M.; Mahna, N.; Ramesh, K.; Bandehagh, A.; Mazzuca, S. Ion homeostasis, osmoregulation, and physiological changes in the roots and leaves of pistachio rootstocks in response to salinity. *Protoplasma* **2018**, *255*, 1349–1362. [CrossRef] [PubMed]
43. Rahneshan, Z.; Nasibi, F.; Moghadam, A.A. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. *J. Plant Interact.* **2018**, *13*, 73–82. [CrossRef]

44. Li, Y.; He, N.P.; Hou, J.H.; Li, X.; Liu, C.C.; Zhang, J.H.; Wang, Q.F.; Zhang, X.M.; Wu, X.Q. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Front. Ecol. Evol.* **2018**, *6*, 64–73. [CrossRef]
45. Xie, Z.C.; Chu, Y.K.; Zhang, W.J.; Lang, D.Y.; Zhang, X.H. *Bacillus pumilus* alleviates drought stress and increases metabolite accumulation in *Glycyrrhiza uralensis* Fisch. *Environ. Exp. Bot.* **2019**, *158*, 99–106. [CrossRef]
46. Liu, J.; Hasanuzzaman, M.; Wen, H.L.; Zhang, J.; Peng, T.; Sun, H.W.; Zhao, Q.Z. High temperature and drought stress cause abscisic acid and reactive oxygen species accumulation and suppress seed germination growth in rice. *Protoplasma* **2019**, *256*, 1217–1227. [CrossRef] [PubMed]
47. Pathania, P.; Rajta, A.; Singh, P.C.; Bhatia, R. Role of plant growth-promoting bacteria in sustainable agriculture. *Biocatal. Agric. Biotechnol.* **2020**, *30*, 101842. [CrossRef]