Halotolerant-\textit{Kocuria rhizophila} (14asp)-Induced Amendment of Salt Stress in Pea Plants by Limiting Na$^+$ Uptake and Elevating Production of Antioxidants

Amir Abdullah Khan 1, Tongtong Wang 1, Tayyaba Hussain 2, Amna 2, Fayaz Ali 3, Fuchen Shi 1,*, Arafat Abdel Hamed Abdel Latef 4, Omar M. Ali 5, Kashif Hayat 6, Shehzad Mehmood 2, Nida Zainab 2, Muhammad Atif Muneer 7, Muhammad Farooq Hussain Munis 2, Mona H. Soliman 8,9, and Hassan Javed Chaudhary 2,*

Abstract: Endophytic bacteria are useful for their safe services in plant growth improvement and for ameliorating abiotic and biotic stresses. Salt-tolerant plant-growth-promoting \textit{Kocuria rhizophila} 14asp (accession number KF 875448) was investigated for its role in pea plants under a saline environment. Salt stress (75 mM and 150 mM NaCl) was subjected to two pea varieties, peas\textregistered 2009 and 9800-10, in a greenhouse under a complete randomized design. Different parameters such as plant growth promotion, relative water content, chlorophyll, antioxidants, and mineral contents were analyzed to elucidate the extent of tolerance persuaded by PGPB (plant-growth-promoting bacteria). Exhibition of adverse effects was noticed in uninoculated varieties. However, inoculation of \textit{K. rhizophila} improved the morphological parameters, antioxidant enzymes, and minimized the uptake of Na$^+$ in plants under various saline regimes. Pea variety 9800-10 exhibited more tolerance than peas\textregistered 2009 in all traits, such as root and shoot length, fresh and dry biomass, chlorophyll contents, and antioxidant enzymes. Our results showed that halotolerant \textit{K. rhizophila} inoculation plays a vital role in enhancing plant growth by interacting ingeniously with plants through antioxidant systems, enduring saline conditions.

Keywords: PGPEs; salinity; pea; antioxidant enzymes; chlorophyll contents

1. Introduction

Salinity is a global problem that directly affects crops by reduced crop growth, production, and yield [1,2]. This salinity issue restricts a plant’s growth within its native habitat and is an ever-increasing problem for arid and semi-arid regions [3,4]. Salinity...
affects more than 10% of arable land and turns it into a desertic/non-agricultural land by making the soil unfavorable for cultivation. In regions of dry climate, 20–27% of soil leads to salinization due to inappropriate irrigation systems all over the world [5]. A UN report revealed that about 50% of the world’s arable land is exposed to salinization [6], with a subsequent economic loss of twelve billion US dollars in the agriculture sector [7].

However, the response of crop plants differ under salinity stress [8]. Most legumes are salt-sensitive plants, but their response towards salinity depends on the climate, soil type, and life stage of plant [9]. However, salt tolerance within legumes, such as the faba bean (Vicia faba), common bean (Phaseolus vulgaris), and Glycine max, are different. Peas, as an essential legume and food plant, are cultivated in many areas of the world. Their grains contain a rich source of carbohydrates, fibers, minerals, vitamins, and proteins [10]. Its productivity may reduce to fifty percent when exposed to salt stress of 100 mM [11]. Nonetheless, its yield was markedly reduced at elevated levels of salt stress [12]. Salinity stress adversely affects growth, development, and yield by limiting metabolic activities of plants, such as osmotic potential, enzymatic activities, and ionic imbalance [13]. Salinity also harms gaseous exchange, as CO$_2$ supply is limited to leaves, which leads to the low rate of photosynthesis, restricted electron transport chain reaction, and production of nascent oxygen ([O]), H$_2$O$_2$, and –OH [14]. [O] is a damaging molecule to plant cells, as it leads to necrosis in the plant [1]. In addition to other impacts, salinity has three significant negative impacts on plant growth and development, which are (a) water scarcity [15], (b) ion toxicity, particularly of Na$^+$ and Cl$^-$ ions, and (c) alterations in nutrient availability, which leads to nutrient imbalance [16]. Storage of excessive Na$^+$ causes disturbance in the metabolic processes where low or high uptake of Na, Ca, and K are required for normal plant functions. In contrast, high uptake and storage of chlorine ions may harm the photosynthetic activity of plants [17]. Salinity restricts plant development and growth by causing damage via specific ion toxicity [18], K$^+$-induced changes in enzymes [19], and ROS production [20]. The damage may vary from leaves to roots at different times. However, injury in floral parts is decisive after exposure to a saline environment for few weeks [21]. This exposure results in more K$^+$ depletion from the roots, producing ROS in root cells after a short time [22]. Due to these circumstances, the root cells may die due to programmed cell death [23].

The plant protects itself from abiotic stresses by utilizing an antioxidant enzyme system, such as ascorbate, glutathione, tocopherols, superoxide dismutase (SOD), peroxidase, catalase (CAT), glutathione peroxidase, and ascorbate–glutathione [24]. Cells act as production houses of enzymes [25]. Synthesis and accumulation of compounds, including soluble sugars and proline, occur in a plant’s cell cytoplasm during salt stress [26,27], which helps in the survival of plants by easing the salinity stress. Some studies show that storage of these compounds is not because of salt stress, as in the cases of sugar beet [28] and Cynara cadunculus [29], where the osmotic adjustment occurred because of organic ions and not the inorganic ones.

Bacteria that dwell inside the plant and enhance its growth and survival by making it tolerant to abiotic and biotic stress are called plant-growth-promoting endophytes [30,31]. PGPE bacterial colonies dwell inside plants without causing any noticeable symptoms [32]. This is why PGPEs are eco-friendly substitutes for chemical-based agricultural practices. Application of PGPEs as a bioinoculant results in PGP and biomass improvement; abi-otic stress tolerance has been carried into consideration in numerous studies [31,33–36]. ACC-deaminase-producing PGPEs alleviate salt stress induced via high production of ethylene as these bacteria can degrade ACC into ammonia and alpha-ketobutyrate [37,38]. ACC-deaminase-producing bacteria promote the development of the plant and alleviate ethylene-induced growth repression [39]. Plant growth promotion was noticed via enhanced production of siderophores, antioxidants, proline, and hydrogen cyanide. Phosphate solubilization and indole acetic acid also increases the chlorophyll content and growth parameters, and subsequently increases the yield [39]. Many studies [35,36] show that ACC-producing bacteria positively alleviate salinity stress in rice and wheat, respectively. In rice plants grown under salinity and boron stress, the chlorophyll contents were
Agronomy 2021, 11, 1907

less compromised than in the uninoculated ones [35]. Reduction in chlorophyll content indicates the plant is under stress conditions and hence affects the process of photosynthesis. The decrease in photosynthetic rate is associated with excessive sodium and chloride ion accumulation, directly affecting plant growth [36,40].

Endophytic bacteria, in conjunction with the host plant’s roots, maintain and change the uptake of nutrients from the soil and balance phytohormones with the release of defensive metabolites from the root [41,42]. Khan et al. [35] found in their study that when B. pumilus was inoculated to rice under salinity and boron stress, it limited uptake of toxic ions and increased production of antioxidants. A small number of microbes, such as Bacillus, Burkholderia, Pseudomonas, Pantoea, and Rhizobium, have been used for providing tolerance against abiotic stresses, including drought, salinity, and heat in different crops [43–47].

Vegetables in Pakistan are irrigated by water that is abundant with different salts that reduce the quality and limit the yield of the crop. As with most other vegetables, peas are irrigated with saline water, which affects the yield and plant physiology. Using PGPEs to ameliorate salinity stress is a highly feasible, eco-friendly, and widely accepted approach compared to conventional breeding and recombinant DNA technology. PGPE, i.e., K. rhizophila, isolated by Mufti et al. [48] from Oxalis corniculata, showed a positive role in the biosorbance of Cd and Cr ions in an aqueous medium [49]. Hussain et al. [50] have reported that K. rhizophila, in synergy with citric acid as a chelator, helps to significantly increase the net biomass of soybean under multiple metal stresses and enhance the uptake of heavy metals. K. rhizophila is a plant-growth-promoting bacteria with a 16 mm positive zone for phosphate solubilization, 0.36 mg/L of IAA, and ammonia production [36].

In our experiment, K. rhizophila an ACC-producing, halotolerant and plant-growth-promoting bacteria (Supplementary Tables S1 and S2) [36], was evaluated against two pea cultivars, peas2009 and 9800-10, to alleviate salt stress under greenhouse conditions. Our study shows, with novelty, that inoculation of K. rhizophila improves the morphological parameters and antioxidant enzymes of plants, and most importantly, minimizes the uptake of Na⁺, under various saline regimes.

2. Materials and Methods

K. rhizophila (GenBank number KF875448) strain was taken from the PMI Laboratory, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. This strain was previously isolated by Mufti et al. [48] from the leaves of Oxalis corniculata.

2.1. Bacteria Salt Stress Analysis

Bacterial strain 14asp was subjected to a salt stress regime. The estimation was conducted through population density at different concentrations ranging from 0 to 15% (weight/volume) in LB medium following the protocol of Afridi et al. [36].

2.2. Bacteria Antibiotic Resistance

To check antibiotic resistance, the disk diffusion method [51] was utilized. Briefly, freshly cultured bacterial broth (100 µL) was swabbed on agar plates with selective antibiotic discs and incubated at 37 °C overnight. The next day, the inhibition zones were measured, and obtained values were further characterized as sensitive, resistant, or intermediate against specific antibiotics using the Kibry-Baurer chart.

2.3. Bacterial Growth Conditions and Pea Seed Inoculations

K. rhizophila was grown in LB medium according to the procedure of Ahmad et al. [52]. Briefly, the bacterial strain was cultured on an incubator at our laboratory (Sun Gene GmbH, Innova 4430, Edison, NJ, USA) for 48 h. After 48 h the growth was adjusted to 1 OD to achieve a culture of the same population density. Pea seeds, i.e., peas2009 and 9800-10 obtained from the National Agriculture Research Center Islamabad (NARC), were sterilized using 75% ethanol and mercuric chloride for 5 and 1 min, respectively, and then washed with distilled water 3–5 times [52]. Surface-sterilized pea seeds were soaked in
previously prepared bacteria culture for 3–4 h, while control seeds were soaked in distilled water [53].

2.4. Experimental Setup

The soil used in this experiment was collected from the warehouse of Quaid-i-Azam University, Islamabad, located between 33.14° N latitudes and 73.13° E longitudes. The topsoil was collected, air-dried, sieved with a 2 mm sieve, mixed with sands in a 3:1 ratio, and autoclaved at a temperature of 121 °C for 20 min [54]. The soil was analyzed for electrical conductivity (EC) (0.14 dS m⁻¹), pH (8.1), and soil organic matter (0.76%). The total available N, P, and K contents were 49.14 mmol/kg, 20 mm/kg, and 3.44 mm/kg. The nature of the soil was clay type with 26%, 33%, and 46% of clay, sand, and silt, respectively. Pots were filled with 1.5 kg autoclaved soil. A total of five treatments were made with three replicates in each with or without *Kocuria* inoculation. In each treatment, eight sterilized seeds at a depth of 2 cm pot were sown. The pea plants were later thinned after two weeks of germination, and only three healthy plants per pot were left, which were carefully watered (50 mL/pot) daily [55]. The pots were kept in the greenhouse under semi-control conditions (temperature: 20 to 37 °C ± 4 °C; a photoperiod of 10 h:14 h (light:dark) with a light intensity of 80 µM S⁻¹ m⁻² and relative humidity of 65 ± 10%). The NaCl treatments used in the current experiment were 0, 75, and 150 mM. Salt stress was given in aliquots of 25 mM daily until reaching the final concentration [56]; for each variety, five treatments were applied (Table 1).

Table 1. Treatment designs of experiment.

| Treatment | NaCl Concentration | Inoculation |
|-----------|--------------------|-------------|
| Control   | ×                  | ×           |
| T1        | 75 mM              | ×           |
| T2        | 150 mM             | ×           |
| T3        | ×                  | ✓           |
| T4        | 75 mM              | ✓           |
| T5        | 150 mM             | ✓           |

2.5. Plant Growth Parameters

After 50 days of exposing the plants to salinity stress, plants were harvested and cleaned from soil particles and debris using tap water. A standard measuring scale and weighting machine was used to measure the length of fresh, dry biomass of roots and shoots of all plants.

2.6. Leaf Relative Water Content (RWC)

Relative water content was determined after harvesting leaves. Briefly, leaves’ fresh weight (FW) was measured. The leaves were then immersed in distilled water for 24 h. Thereafter, fully turgid leaves were re-weighed and turgid weight (TW) was measured. Finally, the leaves were oven-dried at 70 °C for 72 h, until constant dry weight (DW) of leaves was recorded. Relative water content was calculated according to Balestri et al. [57]. RWC was estimated by using the following formula:

\[
RWC = \frac{FW - DW}{TW - DW} \times 100
\]

2.7. Estimation of Leaf Chlorophyll Content

Leaf chlorophyll content was estimated after exposure of plants to salinity stress for eight weeks by following the protocol of Shanker et al. [58]. Briefly, a homogenous mixture of leaves was prepared in DMSO and the readings were calculated via a spec-
trophotometer at different wavelengths, i.e., 480, 649, and 665 nm, respectively. Chlorophyll contents were estimated by applying the given formulas.

$$\text{Chl (a)} = 12.47A_{665.1} - 3.62A_{649.1}$$

$$\text{Chl (b)} = 25.06A_{649.1} - 6.5A_{665.1}$$

$$C_x + c = \frac{(1000A_{480} - 1.29Ca - 53.78Cb)}{220}$$

2.8. Antioxidant Assays in Peas

Antioxidant enzyme assays of SOD, POD, and CAT in fresh pea leaves were determined according to the protocols of Lum [59] as well as Khan et al. [35], respectively.

2.9. Proline Assay in Peas

Proline content in fresh leaves was estimated by following the protocol of Bates et al. [60]. Briefly, 0.5 g of fresh leaves were ground in 4 mL of sulfosalicylic acid (3%) and kept overnight at 5 °C. The next day, centrifugation at 3000 rpm/5 min of the mixture was carried out, followed by adding 2 mL acid ninhydrin. The mixture was heated and after cooling down it was extracted in 4 mL toluene. Optical density was noted at 520 nm and total proline was estimated by the following equation ($k = 17.52$ and dilution factor = 2).

$$\text{Proline} \ \mu g/g = k \ \text{value} \times \text{dilution factor} \times \text{absorbance/fresh sample wt}$$

2.10. Mineral Analysis in Pea Shoots

Minerals such as sodium, potassium, calcium, and magnesium in the pea shoot were determined by following the protocol in [35]. Briefly, a digestion mixture of HNO$_3$ and HCL$_4$ was prepared in a 3:1 v/v ratio. One gram of dried pea plant shoots were digested overnight in an 8 mL digestion mixture. Later, flasks with the digestion mixture were placed on a heat plate until brown-colored fumes turned white. The mixture was cooled down and diluted to 40 mL, followed by filtration via Whatman 42 filter paper. The filtrate was further analyzed by an atomic absorption spectrophotometer.

2.11. Recovery of Inoculated Bacteria

Plants inoculated with *K. rhizophila* after harvesting were washed, surface-sterilized with autoclaved distilled water and cut into small pieces, and finally homogenized in autoclaved water. Later on, the pea plant mixture was poured into nutrient plates. The grown colonies were matched with the control the next day by comparing morphological appearance, gram staining, and antibiotic resistance assay by following the disc diffusion method. Structural formations recognized developing colonies, staining, and antibiotic resistance using the disc diffusion method [36]. Supplementary Table S3 shows the zone of inhibition made by selected antibiotics against 14asp.

2.12. Statistics

For statistical analysis of data, statistics software (Statistix version. 8.1) was used. One-way ANOVA was calculated to conclude the significant value ($p < 0.05$) between the means using Fisher’s least significant difference (LSD) procedure. R plot software was used to carry out PCA (principal component analysis) and Pearson’s correlation of all the data.

3. Results

3.1. Bacteria Salt Tolerance

*K. rhizophila* showed tolerance against salinity at different NaCl levels ranging from five to fifteen percent. The strain grew very well on DF media supplemented with or without nitrogen, which indicates positive results for ACC deaminase activity. Further, the strain was assayed quantitatively for ACC deaminase activity. A comparison was made under control, and 15% of NaCl concentration shows that *K. rhizophila* utilizes ACC
as a nitrogen source by involving ACC deaminase enzymes. However, the level of ACC deaminase was higher in the control than in the stressed one (Figure 1).

![Graph showing ACC deaminase activity of K. rhizophila 14asp under control and 15% NaCl stress conditions.](image)

**K. rhizophila**

Figure 1. ACC deaminase activity of *K. rhizophila* 14asp under control and 15% NaCl stress conditions. The difference above each column is described at *p* value < 0.05 and the bars show error of the mean where *n* = 3.

3.2. Plant Growth Parameters

Pea plant growth attributes such as root length, shoot length, and biomass show significant influence by inoculating *K. rhizophila* (T3) compared to control and uninoculated plants under the saline regime (Table 2). A net decrease in physiological parameters was experienced in salt-treated uninoculated plants (T1, T2) associated with their respective control under similar soil physiochemical characteristics for both pea varieties (peas2009 and 9800-10). The impact of strain 14asp inoculation showed a clear significant increase in growth parameters compared to uninoculated treated plants. In both varieties, the plant growth parameters in inoculated T3 are increased as compared to the control, i.e., In Pea 2009, the SL, RL, and FW are increased by 19.82%, 4.23%, and 3.31%, respectively, compared to the control. Likewise, an increase of 14.83%, 23.67%, and 1.58% in all growth parameters, such as SL, RL, and FW was observed as compared to uninoculated high-salt-treated T2 plants in both varieties. In peas2009 the SL, RL, and FW in T5 increased by 11.65%, 11.73%, and 0.8% as compared to T2-treated plants. While in the 9800-10 T5 group, SL, RL, and FW increased by 12.83%, 11.67%, and 2.07%. Interestingly, no significant difference was observed in DW for both varieties (Table 2).
Table 2. Influence of Kocuria rhizophila inoculation on shoot length (SL), root length (RL), fresh weight (FW), and dry weight (DW) of pea varieties, i.e., peas2009 and 9800-10 grown under salinity stress. All values are means ± standard error of three replicates at the level of p < 0.05. Where T1 = 75 mM NaCl, T2 = 150 mM NaCl, T3 = inoculated with K. rhizophila, T4 = 75 mM of NaCl + K. rhizophila, and T5 = 150 mM NaCl + K. rhizophila. Averages with matching lower-case letters in the same column and upper-case letters in the same row are not significantly different according to the LSD grouping test (p ≤ 0.05).

| Treatments | SL (cm)       | RL (cm)       | FW (g)        | DW (g)        |
|------------|---------------|---------------|---------------|---------------|
|            | peas2009      | 9800-10       | peas2009      | 9800-10       |
| Control    | 18.67 ± 0.16 b| 14.5 ± 0.76 c | 17.5 ± 0.28 b | 23.33 ± 0.66 b|
| T1         | 14.33 ± 0.33 d| 14.05 ± 0.5 d | 11.67 ± 0.66 e| 21.16 ± 0.6 ac|
| T2         | 11.18 ± 0.28 f| 12.33 ± 0.33 f| 11 ± 0.57 f   | 11.4 ± 0.8 e   |
| T3         | 19.46 ± 0.33 a| 15.83 ± 0.16 a| 20.67 ± 0.66 a| 24.67 ± 0.08 a|
| T4         | 16.55 ± 0.28 c| 15.67 ± 0.66 b| 14.33 ± 0.21 c| 22.33 ± 0.33 d|
| T5         | 12.63 ± 0.16 e| 13.83 ± 0.16 e| 12.73 ± 0.8 d | 12.67 ± 0.32 f|

3.3. Leaf Chlorophyll Content

Leaf chlorophyll contents, i.e., chlorophyll a and carotenoids contents, did not decreased in salt-treated uninoculated plants as compared to the control. However, a net decrease in chlorophyll b is noted in uninoculated plants under a saline regime at 150 mM, but inoculation of K. rhizophila 14asp enhanced the chlorophyll b content in both varieties (Figure 2). Inoculation of 14asp helped the 9800-10 variety to enhance chlorophyll b content significantly, by 68% in inoculated plants under 75 mM NaCl.

Figure 2. Effect of K. rhizophila 14asp on electrolyte leakage (a) chlorophyll a, (b) chlorophyll b, (c) carotenoids, and (d) relative water content (RWC) in leaves of pea plants under a saline regime. Each treatment is presented as mean values of triplicate data (n = 3) with standard error (S.E). C represents uninoculated control, T1 (75 mM NaCl), T2 (150 mM NaCl), T3 (inoculated with K. rhizophila), T4 (75 mM NaCl + K. rhizophila), and T5 (150 mM NaCl + K. rhizophila). Different letters on means of treatment shows significant difference at p < 0.05.
3.4. Leaf Relative Water Contents (RWC)

The leaf RWC is not changed in salt-treated plants as compared to the control. However, in inoculated plants exposed to 75 mM concentration, the leaf RWC is significantly higher, with a net increase of up to 73% and 70%, respectively, in inoculated plants exposed to high salinity levels (Figure 2).

3.5. Pea Leaf Antioxidants

Post harvesting, for the plants exposed to salinity stress, the antioxidant activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were evaluated in all treatments (Figure 3). The antioxidants’ (SOD, POD, and CAT) activities in all inoculated plants exposed to salinity are higher than the uninoculated plants of both varieties. The SOD, POD, and CAT activities were increased in both varieties of inoculated plants with increasing salinity concentrations. However, the SOD and CAT activities in 9800-10 variety are higher than peas2009.

![Figure 3](image-url)

**Figure 3.** Effect of *K. rhizophila* 14asp on antioxidants (a) Catalase, (b) peroxidase, (c) proline content, and (d) superoxide dismutase activities in leaves of pea plants under a saline regime. Each treatment is presented as mean values of triplicate data (*n* = 3) with standard error (S.E). C represents uninoculated control, T1 (75 mM NaCl), T2 (150 mM NaCl), T3 (inoculated with *K. rhizophila*), T4 (75 mM NaCl + *K. rhizophila*), and T5 (150 mM NaCl + *K. rhizophila*). Different letters on means of treatment shows significant difference at *p* < 0.05.

3.6. Proline

The proline content in inoculated T3 plants was not enhanced in the peas2009 variety as compared to the control. However, there was a net increase in 9800-10 inoculated plants at 150 mM NaCl compared to uninoculated 150-mM-treated T2 plants. But there was no significant increase in proline in peas2009 (Figure 3d).
3.7. Inorganic Osmolytes

The effects on inorganic osmolyte concentrations, i.e., of Na\(^+\) and K\(^+\) uptake in inoculated plants, were examined (Table 3). The concentration of K\(^+\), Mg\(^{2+}\), and Ca\(^{2+}\) in plant shoots was decreased with increasing concentration in uninoculated plants. In contrast, the Na\(^+\) uptake was increased in uninoculated plants. However, plants inoculated with K. rhizophila, i.e., T4 and T5, decreased the Na\(^+\) uptake in peas 2009 by 17.54% and 22.94% as compared to uninoculated plants. However, the K\(^+\)/Na\(^+\) ratio in peas 2009 was higher than that of 9800-10 at both 75 mM and 150 mM NaCl concentrations.

Table 3. Influence of K. rhizophilla on Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) ions in shoots under NaCl stress. Each treatment is presented as mean values of triplicate data (n = 3) with standard error (S.E). C represents uninoculated control, T1 (75 mM NaCl), T2 (150 mM NaCl), T3 (inoculated with K. rhizophila), T4 (75 mM NaCl + K. rhizophila), and T5 (150 mM NaCl + K. rhizophila). Different letters on means of treatment show significant difference at p < 0.05.

| Treatments | Na\(^+\) (mg g\(^{-1}\)Dw) | K\(^+\) (mg g\(^{-1}\)Dw) | Ca\(^{2+}\) (mg g\(^{-1}\)Dw) | Mg\(^{2+}\) (mg g\(^{-1}\)Dw) |
|------------|--------------------------|--------------------------|--------------------------|--------------------------|
|            | peas2009 | 9800-10 | peas2009 | 9800-10 | peas2009 | 9800-10 | peas2009 | 9800-10 |
| Control    | 15.84 ± 0.06 c | 16.97 ± 0.03 f | 17.18 ± 0.16 e | 20.68 ± 0.02 d | 18.58 ± 0.9 c | 18.97 ± 0.01 a | 18.47 ± 0.02 b | 28.88 ± 0.01 d |
| T1         | 18.54 ± 0.01 b | 19.68 ± 0.03 e | 17.18 ± 0.15 f | 20.78 ± 0.08 c | 18.91 ± 0.5 b | 17.32 ± 0.1 e | 10.98 ± 0.12 e | 37.12 ± 0.02 e |
| T2         | 23.94 ± 0.02 a | 24.26 ± 0.06 a | 17.83 ± 0.13 d | 19.36 ± 0.06 f | 18.95 ± 0.9 a | 17.85 ± 0.08 d | 10.09 ± 0.16 f | 40.01 ± 0.02 b |
| T3         | 14.05 ± 0.01 e | 14.26 ± 0.09 d | 19.47 ± 0.07 a | 21.59 ± 0.26 b | 18.07 ± 0.13 d | 18.37 ± 0.02 c | 19.35 ± 0.38 a | 45.64 ± 0.03 a |
| T4         | 12.83 ± 0.08 f | 20.32 ± 0.05 c | 20.85 ± 0.05 a | 21.91 ± 0.04 a | 17.49 ± 0.04 f | 18.94 ± 0.08 a | 17.48 ± 0.24 d | 27.86 ± 0.1 c |
| T5         | 15.31 ± 0.01 cd | 22.91 ± 0.02 b | 20.33 ± 0.02 b | 20.51 ± 0.01 e | 17.87 ± 0.37 e | 18.61 ± 0.2 b | 12.08 ± 0.79 c | 27.66 ± 0.02 f |

3.8. Principal Component Analysis and Pearson’s Correlation

The principal component analysis clusters the input and response variables into diverse groups on the basis of correlations among them. PCA in the present research separated plant variables of both varieties of pea into separate groups under K. rhizophila inoculation. PCA was also performed to compare treatments of pot experiment for their accumulative effect on plant response traits. In both varieties the PCA divided all five treatments into distinct divisions, indicating dissimilar effects of these treatments from each other (Figure 4a,b). Root length, shoot length, fresh weight, and dry weight were grouped together, showing the enhanced response of these traits under normal and stress conditions as well with inoculation of bacteria (Figure 4a,b). Chlorophyll pigments, including chlorophyll a, b, and carotenoids, were clustered together, implying their similar trend of increasing response under bacterial strain and salinity stress. The various growth, chlorophyll pigments, and antioxidant enzymes activity of plants were correlated positively or negatively by inoculation of bacteria under salt stress (Figure 5). Furthermore, the relative impact of each treatment on the positive and negative regulation of numerous plant attributes is depicted in this diagram. The Pearson correlation coefficient discloses a substantial negative correlation among plant growth parameters and salt stress. A strong correlation was observed between root, shoot length, and chlorophyll contents of seedlings, as shown by the red color of Figure 4. In the same way, a negative correlation was observed among antioxidant enzymes and growth variables of seedlings, as shown by the blue color of Figure 4.
Figure 4. The PCA biplot shows the effect of *K. rhizophilla* inoculation on correlation among different growth attributes and treatments of pea varieties under 75 mM and 150 mM of NaCl stress. (a) The correlation among treatments of variety peas2009. (b) The effect of 14asp on correlation among growth variables of peas2009. (c) The correlation among treatments. (d) The correlation among variables of seedlings of variety 9800-10.
4. Discussion

This study shows the potential of halotolerant *K. rhizophila* as an efficient PGPE that enhances the growth of pea plants under various saline regimes. The current research has shown that pea plants inoculated with *K. rhizophila* had higher morphological attributes, such as biomass, shoot, root length, chlorophyll contents, RWC, antioxidants, K\(^+\)/Na\(^+\) ratios, and antioxidant enzymes, as compared to uninoculated plants. *K. rhizophila* minimized the adverse effects of salinity in inoculated plants (Table 2 and Figures 2 and 3). Previously, the role of PGPEs in stimulating the growth, nutrient uptake, symbiosis, and stress tolerance of wheat, alfalfa, tomatoes, and chickpea plants under salt stress had been reported [36,61–63]. All these findings show the positive role of PGPEs in tolerating salinity stress by increasing plant growth and antioxidant enzymes.
imized the adverse effects of salinity in inoculated plants (Tables 2 and 3; Figures 2 and 3). Previously, the role of PGPEs in stimulating the growth, nutrient uptake, symbiosis, and stress tolerance of wheat, alfalfa, tomatoes, and chickpea plants under salt stress had been reported [36,61–63]. All these findings show the positive role of PGPEs in tolerating salinity stress by increasing plant growth and antioxidant enzymes.

In our current work, plant growth parameters such as root length, shoot length, and biomass showed significant influence by *K. rhizophilla* inoculation (T3) as compared to the control. A net decrease was observed in salt-treated plants (i.e., 75 mM and 150 mM) related to their respective control in the same soil having similar physiochemical characteristics for both pea varieties (peas2009 and 9800-10). Tomato plants inoculated with *Sphingomonas* sp. LK11, an endophytic strain, promoted growth parameters under a saline regime [62]. In the same way, *Kocurria* sp., when inoculated in two varieties of wheat plant, i.e., pasban and khirman, showed a net increase in biomass and other growth parameters such as root length, shoot length, fresh biomass, and dry weight under salinity stress, which is in accordance with our findings [36]. Considering all these research findings, the role of PGPEs is very evident in plant growth promotion and its ability to overcome salinity stress.

PGPEs increase water uptake in plants exposed to salinity stress, thereby increasing the photosynthesize rate suppressed by saline conditions [64]. In our experiments, the leaf RWC is decreased in salt-treated plants. But in inoculated plants, it is significantly higher than in uninoculated ones. There was an increase of 77.26% and 65.66% in T4 plants of peas2009 and 9800-10, respectively, compared to T1 (75 mM NaCl) uninoculated plants of both varieties. The same pattern of higher leaf water potential with a net increase of 73% and 70% was found in T5 as compared to T2. These results are in accordance with [61]. Alfalfa plants inoculated with endophytic bacteria showed an increase in water absorption and relative water content under salt stress; the reason was an increase in root length and weight compared to uninoculated plants. Inoculation of plants with bacteria changes the lateral root system and hence increases RWC [61]. Our results are in agreement with results previously noted in pea plants [65], alfalfa [66], and maize [67].

Plants under salinity stress have decreased chlorophyll activity as chlorophyllase activity becomes enhanced in salt conditions, which destroys pigment proteins, eventually reducing chlorophyll content in plants [68,69]. Our results showed that inoculation with *K. rhizophila* protects chlorophyll pigments in both varieties and saved plants from the negative effect of salinity compared to uninoculated pea plants. Our findings are in agreement with the findings observed in lettuce and basil plants [70,71]. The reason behind the good morphological parameters was the higher amount of chlorophyll in inoculated plants which directly or indirectly helps in higher uptake of Fe, Mg, and N [72], and also restricts ethylene production, alleviating its drastic effects on plants [73].

Plants overcome the adverse effects of stress by producing antioxidant enzymes. SOD, POD, and CAT enzymes have low molecular weight but efficiently overcome salt stress by various mechanisms, making the plant tolerant against salt stress [74]. However, many plant species cannot produce enough antioxidants that can adequately overcome the drastic effects of salinity. In past findings, plants such as maize, gladiolus, and chickpea mitigate salinity stress with the help of SOD, POD, CAT, and proline [54,75,76], respectively. In our experiments, the antioxidant activities in all inoculated plants were higher than in the uninoculated plants of both varieties when exposed to salinity.

Endophytes have a prominent role in minimizing the negative impacts of salinity via the accumulation of osmolytes and antioxidant enzymes. Osmolytes and antioxidants enzymes contribute to osmotic adjustment and act as scavengers against free radicals. Endophytic bacterial strains, i.e., *Bacillus* sp. and *Arthrobacter* sp., have previously been reported to have a promising role in accumulating proline content in pepper (*Capsicum annuum* L.) plants under stress conditions [75]. The 9800-10 pea variety shows the same increase in proline content at 150-mM-inoculated plants compared to uninoculated plants. The increased amount of proline in pea variety 9800-10 as compared to variety peas2009 might be the possible
reason for making it more tolerant than peas2009 (Figure 2C), showing a positive role of proline in salt tolerance [76].

*K. rhizophila*-induced amendment of salt stress in pea plants was directly linked to efficient uptake of nutrients and extrusion of toxic ions, including Na. Pea plants inoculated with *K. rhizophila* show decreased Na ion uptake as compared to uninoculated plants in both varieties. The results also show that inoculation with bacteria has a net positive effect to increase root growth and K⁺ uptake respectively, but decreased Na⁺ under saline conditions. These results are in agreement with [21]. When inoculated with different bacterial strains, plants under the saline conditions demonstrated a change in Na⁺ and K⁺ selectivity and a decrease in uptake and transportation into the whole plant body [77]. Moreover, previous studies have shown that bacterial inoculation in plants could have a promising role in root growth, nutrient absorption, producing organic acids, reducing pH, and siderophore exudation in inoculated plants’ rhizosphere [78,79]. Recently, application of *D. natronolimanae* to wheat seedlings under saline conditions showed that the oxidative damage was decreased in plants by enhancing the expression of important proteins, i.e., HKT, SOS, HAK, and NHX. These are commonly known transport proteins and were saved from the negative effects and compartmentation of toxic ions [80]. Higher intake and accumulation of Na⁺ affect plants by inducing osmotic and ionic stress, contributing to oxidative damage and negatively affecting the K⁺ intake by salt-stressed plants. This alteration in nutrient uptake eventually effects the K⁺/Na⁺ ratio. However, pea plants inoculated with *K. rhizophila* showed an improvement in K/Na ratio, thus protecting from Na⁺ accumulation in plants affecting transport proteins. Moreover, low values of K⁺/Na⁺ enhances the plant susceptibility and osmotic potential alterations [81]. K⁺ is an important macro-molecule which helps in movements across the stomata, enzyme activation and stress tolerance [82].

A combined PCA analysis of all plant parameters was performed in the present research. All plant growth parameters were gathered that display improved response of these attributes under normal and stress conditions, as well through inoculation of strain 14asp. The multivariate analysis estimates the variance and correlation of various response variables with input factors [82]. Multivariate analysis such as PCA is being applied most recently to uncover trends and linkages among data sets [83]. In the current study, the PCA biplot disclosed that the general effect of strain 14asp and salt stress on plant responses varied from each other and the control. Normally, employed statistical estimations may not be sufficient to demonstrate significant differences between treatments; however, PCA may provide an accurate and in-depth understanding of dataset differences [82].

5. Conclusions

In the current study, growth and nutrient uptake was affected in uninoculated pea plants grown under saline conditions, which resulted in specific alterations in physiological and biochemical characteristics. Nevertheless, the application of *K. rhizophila* significantly enhanced the growth parameters of pea plants such as plant height, fresh weight, dry weight, and chlorophyll pigments (Chl a, Chl b, and carotenoids) by modulating the antioxidant systems. Bacterial inoculation also showed a conclusive role in the lower uptake of toxic ions; specifically, a decrease in Na uptake, protecting both varieties from oxidative damage. However, pea variety 9800-10 inoculated with *K. rhizophila* showed enhanced salt tolerance, growth parameters, and higher antioxidants activities than inoculated peas2009. Our results suggest that *K. rhizophila* plays a promising role in ameliorating salinity-induced stress, and could be a sustainable solution in the future.

**Supplementary Materials:** The following are available online at [https://www.mdpi.com/article/10.3390/agronomy11101907/s1](https://www.mdpi.com/article/10.3390/agronomy11101907/s1), Table S1: Morphological and Biochemical Characterization and Plant growth promotion activities of *K. rhizophila* Isolated from *Oxalis corniculata* (Mufti et al., 2015). Table S2: Plant growth promoting assays of *K. rhizophila*. Table S3: Testing susceptibility of 15 selected antibiotics disc against *Kocurria rhizophila* 14asp.
Author Contributions: Data curation, M.A.M.; Formal Analysis, A.A.K., S.M., A. and N.Z.; Funding Acquisition, A.A.H.A.L., O.M.A., F.S. and M.H.S.; Investigation, A.A.K.; Methodology, A.A.K., A., T.W., T.H., F.A. and K.H.; Project Administration, H.J.C. and F.S.; Resources, M.F.H.M.; Supervision, H.J.C. and F.S.; Writing—original draft, A.A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Taif University Researchers Supporting Project (TURSP-2020/81), Taif University, Taif, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

Acknowledgments: This research was funded by the Taif University Researchers Supporting Project number (TURSP-2020/81), Taif University, Taif, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Shaukat, M.; Wu, J.; Fan, M.; Hussain, S.; Yao, J.; Serafim, M.E. Acclimation improves salinity tolerance capacity of pea by modulating potassium ions sequestration. *Sci. Hortic.* 2019, **254**, 193–198. [CrossRef]
2. Pandolfi, C.; Mancuso, S.; Shabala, S. Physiology of acclimation to salinity stress in pea (*Pisum sativum*). *Environ. Exp. Bot.* 2012, **84**, 44–51. [CrossRef]
3. Shannon, M.C. In quest of rapid screening techniques for plant salt tolerance. *HortScience* 1979, **14**, 587–589.
4. Qados, A.M.S.A. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *J. Saudi Soc. Agric. Sci.* 2011, **10**, 7–15.
5. Ghassemi, F.; Jakeman, A.J.; Nix, H.A. *Salinisation of Land and Water Resources: Human Causes, Extent, Management and Case Studies*; CAB International: Wallingford, UK, 1995.
6. Hernández, J.A.; Rio, L.A.D.; Sevilla, F. Salt stress-induced changes in superoxide dismutase isozymes in leaves and mesophyll protoplasts from *Vigna unguiculata* (L.) Walp. *New Phytol.* 2006, **126**, 37–44. [CrossRef]
7. Shabala, S. Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann. Bot.* 2013, **112**, 1209–1221. [CrossRef]
8. Hampson, C.R.; Simpson, G.M. Effects of temperature, salt, and osmotic potential on early growth of wheat (*Triticum aestivum*). I. Germination. *Can. J. Bot.* 1990, **68**, 524–528. [CrossRef]
9. Cordovilla, M.P.; Ocana, A.; Ligero, F.; Lluch, C. Salinity effects on growth analysis and nutrient composition in four grain legumes–*rhizobium* symbiosis. *J. Plant Nutr.* 1995, **18**, 1595–1609. [CrossRef]
10. Nikolopoulou, D.; Grigorakis, K.; Stasini, M.; Alexis, M.N.; Iliadis, K. Differences in chemical composition of field pea (*Pisum sativum*) cultivars: Effects of cultivation area and year. *Food Chem.* 2007, **103**, 847–852. [CrossRef]
11. Rao, G.V.S.; Jehansen, C.; Rao, J.V.D.K.K.; Jana, M.K. Salinity Tolerance in F1 Hybrids of Pigeonpea and a Tolerant Wild Relative. *Crop Sci.* 1990, **30**, 785–788.
12. Najafi, F.; Khavarinejad, R.A.; Rastgarjazii, F.; Sticklen, M. Growth and Some Physiological Attributes of Pea (*Pisum sativum* L.) As Affected by Salinity. *Pak. J. Biol. Sci.* 2007, **10**, 2752–2755.
13. Vinocur, B.; Altman, A. Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. *Curr. Opin. Biotechnol.* 2005, **16**, 123–132. [CrossRef] [PubMed]
14. Mateo, A. *LESION SIMULATING DISEASE 1 Is Required for Acclimation to Conditions That Promote Excess Excitation Energy*. *Plant Physiol.* 2004, **136**, 2818–2830. [CrossRef]
15. Termaat, A.; Munns, R. Use of Concentrated Macronutrient Solutions to Separate Osmotic from NaCl-specific Effects on Plant Growth. *Funct. Plant Biol.* 1986, **13**, 509–522. [CrossRef]
16. Silberbush, M.; Ben-Asher, J. Simulation study of nutrient uptake by plants from soilless cultures as affected by salinity buildup and transpiration. *Plant Soil* 2001, **233**, 59–69. [CrossRef]
17. Xu, G.; Magen, H.; Tarchitzky, J.; Kafkafi, U. Advances in Chloride Nutrition of Plants. *Adv. Agron.* 2000, **68**, 97–110.
18. Cuin, T.A.; Shabala, S. Compatible solutes reduce ROS-induced potassium efflux in Arabidopsis roots. *Plant Cell Environ.* 2007, **30**, 875–885. [CrossRef]
19. Chen, Z.; Pottosin, I.I.; Cuin, T.A.; Fuglsang, A.T.; Tester, M.; Jha, D.; Zepeda-Jazo, I.; Zhou, M.; Palmgren, M.G.; Newman, I.A.; et al. Root Plasma Membrane Transporters Controlling K⁺/Na⁺ Homeostasis in Salt-Stressed Barley. *Plant Physiol.* 2007, **145**, 1714–1725. [CrossRef]
20. Sairam, R.K.; Srivastava, G.C. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Sci.* 2002, **162**, 897–904. [CrossRef]
21. Munns, R.; Tester, M. Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Biol.* 2008, **59**, 651–681. [CrossRef]
22. Demidchik, V.; Shabala, S.N.; Davies, J.M. Spatial variation in H₂O₂ response of *Arabidopsis thaliana* root epidermal Ca²⁺ flux and plasma membrane Ca²⁺ channels. *Plant J. Cell Mol. Biol.* 2007, **49**, 377–386. [CrossRef]
50. Hussain, A.; Amna; Kamran, M.A.; Javed, M.T.; Hayat, K.; Farooq, M.A.; Ali, N.; Ali, M.; Manghwar, H.; Jan, F. Individual and combinatorial application of *Kocuria rhizophila* and citric acid on phytoextraction of multi-metal contaminated soils by *Glycine max* L. *Environ. Exp. Bot.* 2019, 159, 23–33. [CrossRef]

51. Din, B.U.; Sarfraz, S.; Xia, Y.; Kamran, M.A.; Javed, M.T.; Sultan, T.; Munis, M.F.H.; Chaudhary, H.J. Mechanistic elucidation of germination potential and growth of wheat inoculated with exopolysaccharide and ACC-deaminase producing *Bacillus* strains under induced salinity stress. *Ecotoxicol. Environ. Saf.* 2019, 183, 109466.

52. Ahmad, I.; Akhtar, M.J.; Asghar, H.N.; Ghafoor, U.; Shahid, M. Differential Effects of Plant Growth-Promoting Rhizobacteria on Maize Growth and Cadmium Uptake. *J. Plant Growth Regul.* 2015, 35, 303–315. [CrossRef]

53. Adesemoye, A.O.; Obini, M.; Ugoji, E.O. Comparison of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. *Braz. J. Microbiol.* 2008, 39, 423–426. [CrossRef] [PubMed]

54. Khan, N.; Bano, A. Role of plant growth promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation. *Int. J. Phytoremediation* 2016, 18, 211–221. [CrossRef] [PubMed]

55. Chen, Z.; Sheng, X.; He, L.; Huang, Z.; Zhang, W. Effects of root inoculation with bacteria on the growth, Cd uptake and bacterial communities associated with rape grown in Cd-contaminated soil. *J. Hazard. Mater.* 2013, 244, 709–717. [CrossRef]

56. Noreen, S.; Ashraf, M. Alliation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid: Growth and photosynthesis. *Pak. J. Bot.* 2008, 40, 1657–1663.

57. Balestri, M.; Bottega, S.; Spanò, C. Response of *Pteris vittata* to different cadmium treatments. *Acta Physiol. Plant.* 2014, 36, 767–775. [CrossRef]

58. Shankar, V.; Kumar, D.; Agrawal, V. Assessment of antioxidant enzyme activity and mineral nutrients in response to NaCl stress and its amelioration through glutathione in chickpea. *Appl. Biochem. Biotechnol.* 2016, 178, 267–284. [CrossRef]

59. Lum, M.; Hanafi, M.; Rafii, Y.; Akmar, A. Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *J. Anim. Plant Sci.* 2014, 24, 1487–1493.

60. Bates, L.S.; Waldren, R.P.; Teare, I. Rapid determination of free proline for water-stress studies. *Plant Soil* 1973, 39, 205–207. [CrossRef]

61. Ansari, M.; Shekari, F.; Mohammadi, M.H.; Juhos, K.; Végvári, G.; Biró, B. Salt-tolerant plant growth-promoting bacteria enhanced salinity tolerance of salt-tolerant alfalfa (*Medicago sativa* L.) cultivars at high salinity. *Acta Physiol. Plant.* 2019, 41, 195. [CrossRef]

62. Halo, B.A.; Khan, A.L.; Waqas, M.; Al-Harrasi, A.; Hussain, J.; Ali, L.; Adnan, M.; Lee, I.-J. Endophytic bacteria (*Sphingomonas* sp. LK11) and giberrellin can improve *Solanum lycopersicum* growth and oxidative stress under salinity. *J. Plant Interact.* 2015, 10, 117–125. [CrossRef]

63. Abd_Allah, E.F.; Alqarawi, A.A.; Hashem, A.; Radhakrishnan, R.; Al-Huqail, A.A.; Al-Otibi, F.O.N.; Malik, J.A.; Alharbi, R.I.; Egamberdieva, D. Endophytic bacterium *Kocuria rhizophila* (*BERA 71*) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J. Plant Interact.* 2018, 13, 37–44. [CrossRef]

64. Hashem, A.; Abd_Allah, E.F.; Alqarawi, A.A.; Al-Huqail, A.A.; Wirth, S.; Egamberdieva, D. The Interaction between Arbuscular Mycorrhizal Fungi and Endophytic Bacteria Enhances Plant Growth of *Acacia gerrardi* under Salt Stress. *Front. Microbiol.* 2016, 7, 1089. [CrossRef] [PubMed]

65. Ali, Z.; Ullah, N.; Naseem, S.; Haq, M.I.U.; Jacobsen, H.J. Soil bacteria conferred a positive relationship and improved salt stress tolerance in transgenic pea (*Pisum sativum* L.) harboring Na+ / H+ antiporter. *Turk. J. Bot.* 2015, 39, 962–972. [CrossRef]

66. Bertrand, A.; Dhont, C.; Bifubusa, M.; Chalifour, F.-P.; Drouin, P.; Beauchamp, C.J. Improving salt stress responses of the symbiosis in alfalfa using salt-tolerant cultivar and rhizobial strain. *Appl. Soil Ecol.* 2015, 87, 108–117. [CrossRef]

67. Bano, A.; Fatima, M. Salt tolerance in *Zea mays* (L.) following inoculation with Rhizobium and *Pseudomonas*. *Biol. Fertil. Soils* 2009, 45, 405–413. [CrossRef]

68. Jaleel, C.A.; Sankar, B.; Sridharan, R.; Panneerselvam, R. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. *Turk. J. Biol.* 2008, 32, 79–83.

69. Anower, M.R.; Mott, I.W.; Peel, M.D.; Wu, Y. Characterization of physiological responses of two alfalfa half-sib families with improved salt tolerance. *Plant Physiol.* 2013, 71, 103–111. [CrossRef]

70. Heidari, M.; Golpayegani, A. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *J. Saudi Soc. Agric. Sci.* 2012, 11, 57–61. [CrossRef]

71. Han, H.; Lee, K. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. *Res. J. Agric. Biol. Sci.* 2005, 1, 210–215.

72. Hosseinzadah, F.; Satei, A.; Ramezanpour, M.R. Effects of mycorhiza and plant growth promoting rhizobacteria on growth, nutrients uptake and physiological status (*Calendula officinalis*). *Cellulae officinalis L.* *Middle East J. Sci. Res.* 2011, 8, 947–953.

73. Habib, S.H.; Kausar, H.; Saud, H.M. Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. *BioMed Res. Int.* 2016, 2016, 6284547. [CrossRef]

74. Xu, H.-C.; Tie, C.; Wang, Z.-L.; HE, M.-R. Physiological basis for the differences of productive capacity among tillers in winter wheat. *J. Integr. Agric.* 2015, 14, 1958–1970. [CrossRef]

75. Akram, M.S.; Shahid, M.; Tariq, M.; Azeem, M.; Javed, M.T.; Saleem, S.; Riaz, S. Deciphering Staphylococcus sciuri SAT-17 mediated anti-oxidative defense mechanisms and growth modulations in salt stressed maize (*Zea mays* L.). *Front. Microbiol.* 2016, 7, 867. [CrossRef]
76. Han, Q.-Q.; Lü, X.-P.; Bai, J.-P.; Qiao, Y.; Paré, P.W.; Wang, S.-M.; Zhang, J.-L.; Wu, Y.-N.; Pang, X.-P.; Xu, W.-B.; et al. Beneficial soil bacterium Bacillus subtilis (GB03) augments salt tolerance of white clover. *Front. Plant Sci.* **2014**, *5*, 525. [CrossRef] [PubMed]

77. Sziderics, A.; Rasche, F.; Trognitz, F.; Sessitsch, A.; Wilhelm, E. Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). *Can. J. Microbiol.* **2007**, *53*, 1195–1202. [CrossRef] [PubMed]

78. Younesi, O.; Moradi, A. Effects of plant growth-promoting rhizobacterium (PGPR) and arbuscular mycorrhizal fungus (AMF) on antioxidant enzyme activities in salt-stressed bean (*Phaseolus vulgaris* L.). *Agriculture (Pol’nohospodářstvo)* **2014**, *60*, 10–21. [CrossRef]

79. Volkov, V. Salinity tolerance in plants. Quantitative approach to ion transport starting from halophytes and stepping to genetic and protein engineering for manipulating ion fluxes. *Front. Plant Sci.* **2015**, *6*, 873. [CrossRef] [PubMed]

80. Baset, M.; Shamsuddin, Z.; Wahab, Z.; Marziah, M. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured’ musa’ plantlets under nitrogen-free hydroponics condition. *Aust. J. Crop. Sci.* **2010**, *4*, 85.

81. Dodd, I.C.; Pérez-Alfocea, F. Microbial amelioration of crop salinity stress. *J. Exp. Bot.* **2012**, *63*, 3415–3428. [CrossRef]

82. Bharti, N.; Pandey, S.S.; Barnawal, D.; Patel, V.K.; Kalra, A. Plant growth promoting rhizobacteria *Dietzia natronolimnaea* modulates the expression of stress responsive genes providing protection of wheat from salinity stress. *Sci. Rep.* **2016**, *6*, 34768. [CrossRef]

83. Wu, Q.-S.; Zou, Y.-N.; He, X.-H. Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. *Acta Physiol. Plant.* **2010**, *32*, 297–304. [CrossRef]