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PGPB Improve Photosynthetic Activity and Tolerance to Oxidative Stress in *Brassica napus* Grown on Salinized Soils

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Abstract: Soil salinization, one of the most common causes of soil degradation, negatively affects plant growth, reproduction, and yield in plants. Saline conditions elicit some physiological changes to cope with the imposed osmotic and oxidative stresses. Inoculation of plants with some bacterial species that stimulate their growth, i.e., plant growth-promoting bacteria (PGPB), may help plants to counteract saline stress, thus improving the plant's fitness. This manuscript reports the effects of the inoculation of a salt-sensitive cultivar of *Brassica napus* (canola) with five different PGPB species (separately), i.e., *Azospirillum brasilense, Arthrobacter globiformis, Burkholderia ambifaria, Herbaspirillum seropedicae,* and *Pseudomonas* sp. on plant salt stress physiological responses. The seeds were sown in saline soil (8 dS/m) and inoculated with bacterial suspensions. Seedlings were grown to the phenological stage of rosetta, when morphological and physiological features were determined. In the presence of the above-mentioned PGPB, salt exposed canola plants grew better than non-inoculated controls. The water loss was reduced in inoculated plants under saline conditions, due to a low level of membrane damage and the enhanced synthesis of the osmolyte proline, the latter depending on the bacterial strain inoculated. The reduction in membrane damage was also due to the increased antioxidant activity (i.e., higher amount of phenolic compounds, enhanced superoxide dismutase, and ascorbate peroxidase activities) in salt-stressed and inoculated *Brassica napus*. Furthermore, the salt-stressed and inoculated plants did not show detrimental effects to their photosynthetic apparatus, i.e., higher efficiency of PSII and low energy dissipation by heat for photosynthesis were detected. The improvement of the response to salt stress provided by PGPB paves the way to further use of PGPB as inoculants of plants grown in saline soils.

Keywords: *Brassica napus*; PGPB; salt stress; photosynthesis; oxidative stress

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1. Introduction

The continued increase in land affected by salinization [1] makes it necessary to select crops that are salt tolerant, to avoid the yield loss that would otherwise be associated with high soil salinity. Salt-stressed plants undergo osmotic and oxidative stresses that negatively influence their growth. Photosynthetic activity can be affected by salinity as well [2], e.g., studies by Hnilickova et al. [3] highlighted that *Eruca sativa* (arugula) had lower photosynthetic activity when watered with 100 mM NaCl solution than when water was employed. Generally, in salt-sensitive plants exposed to salinity, the amount of chlorophyll decreases, and the performance of the photosystems and electron transport mechanism

are negatively impacted [4]. This can also involve damage of PSII by reactive oxygen species (ROS) causing a decrease in photosynthetic activity [5]. Photosynthesis is one of the physiological processes that are extremely susceptible to environmental stress, and for this reason, photosynthetic efficiency can be a good biomarker of environmental pressure in stressed plants [6]. In this context, chlorophyll fluorescence imaging can be analyzed to evaluate plant stresses, allowing in vivo analysis that it is non-destructive [7]. Moreover, it allows the evaluation of the heterogeneity in photosynthetic functions throughout a leaf, owing to image analysis of the quantum efficiency of photosystem II (PSII) in plants affected by several stresses, including heavy metals [8,9], salinity [10], pharmaceuticals [11], and emerging contaminants [12].

Brassica napus L. is a very important crop commonly named canola. Its agronomical relevance derives from food use (cultivars containing low quantities of erucic acid and glucosinolate) and from the high amounts of oils, useful for biodiesel production [13]. This species is a glycophyte, which is quite sensitive to the presence of salt in the soil, even though its genotypes can range from sensitive to tolerant [10]. Different approaches can be applied to help canola plants to overcome salt stress, including plant acclimation [10] and seed priming [14]. In addition, the establishment of a mutualistic relationship between plants and plant growth-promoting bacteria (PGPB) [15–20] can be a good strategy to help plants to overcome salt stress.

PGPB [21] are soil bacteria able to improve plant growth directly (by producing beneficial compounds) or indirectly (by inhibiting plant pathogens). Species belonging to the genera *Azospirillum, Burkholderia, Pseudomonas*, and *Enterobacter* have been reported to be PGPB [16,22]. Several beneficial activities have been attributed to them, including the synthesis of osmoprotectants, siderophores, and phytohormones (auxin, cytokinins, gibberellins, and abscisic acid). Some strains can also fix atmospheric nitrogen, solubilize organic and inorganic phosphate, and they can reduce the synthesis of stress ethylene by the plants as a consequence of the activity of the enzyme 1-aminocyclopropane-1-carboxylate (ACC deaminase) [16,23–26].

Despite the growing interest in the use of PGPB to alleviate plant stress to salinity, in the literature, there are few reports concerning the effect of PGPB on physiological and biochemical response in plants exposed to stressful levels of salt [27,28].

The bacterial species, selected for this study, *Azospirillum brasilense* strain CD, *Arthrobacter globiformis* strain CD, *Burkholderia ambifaria* strain PHP7, *Herbaspirillum seropedicae* strain Z67, and *Pseudomonas* sp. strain UW4 share the above-mentioned PGPB characteristics. All these strains produce auxins and polysaccharides [29–33]. Moreover, *B. ambifaria*, *H. seropedicae,* and *Pseudomonas* sp. have ACC deaminase activity [15,33–36], while *A. brasilense* and *A. globiformis* do not possess this activity [20,33], even though they belong to genera in which the ACC deaminase has been detected in other strains [37–39].

The aim of this work was to assess more deeply the effects of these five different PGPB on photosynthetic and antioxidant activities of a salt-sensitive cultivar of *B. napus* exposed to saline conditions. Osmolyte synthesis was also determined.

2. Materials and Methods

2.1. Chemicals and Reagents

The reagents were analytical grade or equivalent and purchased from Merck or Sigma-Aldrich unless otherwise stated. In each set of experiments, all working solutions were prepared immediately prior to use from stock reagents.

2.2. Bacterial Cultures

A. globiformis strain CD was from the collection of the Laboratory of Botany and Phytotechnologies of the Department of Biology of the University of Rome Tor Vergata. *Pseudomonas* sp. strain UW4 was isolated from soil from the campus of the University of Waterloo, Waterloo, Ontario, Canada [40]. *A. brasilense* strain CD, *B. ambifaria* strain PHP7, and *H. seropedicae* strain Z67 are from the collection of Prof. Maddalena Del Gallo of the

Laboratory of Environmental Microbiology, Department of Health, Life and Environmental Sciences of the University of L'Aquila, Italy.

The strains were stored in glycerol solution at −80 °C. The bacteria were grown on tryptic soy broth (TSB) agar plates (0.8% agar, Sigma-Aldrich) at 30 °C. To assess their halotolerance, bacteria were grown in a TSB medium with the following NaCl concentrations: 0 mM, 160 mM, 320 mM, and 640 mM. Bacterial cultures were grown for 120 h at 30 °C at 150 rpm (New Brunswick Orbital Shaker). The OD_{600} was measured every 24 h.

2.3. Plant Growth Conditions

The seeds of canola cultivar Sy Saveo were supplied by Dr. Montanari of the CREA-CIN (Centro di Ricerca per le Colture Industriali), Bologna, Italy. The chosen cultivar synthesized high amounts of oils and low quantities of erucic acid and glucosinolate and was sensitive to salinity [10]. Seed germination tests in presence of both salt and PGPB were performed as follows: the seeds were surface sterilized (70% ethanol for 5 min, followed by 1% NaClO for 1 min). In total, 25 inoculated seeds per replicate (3 replicates per treatment) were placed in Petri dishes with sterile Hoagland's medium [41] (1:10 strength) with agar (0.8% w/v), with the following NaCl concentrations: 0 mM (control), 80 mM (saline), and kept in the dark. Seed germination rates were recorded after 48 h.

Pot experiments: seeds were surface sterilized and sown in plastic pots (4 seeds per pot; pot volume = 0.00212 m^3), containing 375 g of soil (soil characteristics: pH 6.5; dry bulk density 150 kg/m^3; porosity 90% v/v. Soil components: neutral sphagnum peat, perlite (<5%), composted green soil improver). Saline soil (S) was prepared by adding a solution of NaCl to obtain an electrical conductivity (EC) of 8.2 dS/m. Control soil (C) had no saline added (EC = 0.3 dS/m). Pots were irrigated with 100 mL of water (C) or 80 mM of NaCl (S) twice a week. The EC of soils was kept constant up to the end of the experiments. The soil EC was measured according to Santangeli et al. [10].

The bacterial inoculation was performed directly on the seeds after the sowing, by inoculating 1 mL of single strain bacterial solution (1×10^6 CFU) per seed. Experimental groups (3 replicates per group) were divided as follows: (1) non-inoculated seeds in control soil (N.I.C.); (2) non-inoculated seeds in saline soil (N.I.S.); (3) inoculated seeds in control soil (bacterial species names, C); (4) inoculated seeds in saline soil (bacterial species names, S).

Plant growth lasted for 30 days at 23 ± 2 °C and 48 ± 2% relative humidity. the pots were daily moved randomly in the growth chamber, with a photoperiod of 16/8 h, PAR 30 moles photons m^{-2} s^{-1} (lamp: 2× OSRAM, FLUORA t8 36.00 W and 2× OSRAM, LUMILUX Cool Daylight t8 36.00 W).

Plant growth was determined as shoot length and longest root length (cm) (n = 16). The biomass was determined by evaluating the total fresh weight (g. f.w.) of the plants (n = 16). Samples (200 mg f.w.) were frozen with liquid nitrogen and stored at −80 °C until biochemical and physiological analyses, except for the membrane injure index and water content. The frozen samples were homogenized in liquid nitrogen with ceramic mortars and pestles and suspended in solution following the protocols reported below.

2.4. Water Content

Plant water content was determined according to Zeng et al. [42]. Plants (n = 8) were dried at 70 °C for 48 h. The average (%) of water content was calculated according to the following formula:

$$\text{Water content} = [(\text{f.w.} - \text{d.w.})/\text{f.w.}] * 100$$

f.w. = Plant fresh weight
d.w. = Plant dry weight

2.5. Proline Content

Proline was extracted from frozen homogenized samples (50 mg f.w.) (n = 9) and heated at 55 °C for 20 min in 95% ethanol. The quantitative determination of proline was

performed according to the protocol of Santangeli et al. [10], i.e., 500 µL of the extract was added to 1 mL of reaction mixture composed of ninhydrin (2,2-dihydroxyindane-1,3-dione) 1 % (*w*/*v*) dissolved in a mixture of 60% (*v*/*v*) acetic acid and 20% (*v*/*v*) ethanol. The samples, protected from light, were heated at 95 °C for 20 min and then centrifuged for 1 min at 7000× *g*. The proline concentration was evaluated by detecting the absorbance at 520 nm. The proline concentration was calculated according to a curve made with standard solutions of L- Proline ranging from 0.1 to 1 mM (y = 0.0104x + 0.0294; R^2 = 0.99). Data are expressed as nmoles proline /mg f.w.

2.6. Membrane Injury Index (MII)

According to Santangeli et al. [10], the electrical conductivity is measured on fresh plant samples (*n* = 8) to calculate the membrane injury index (MII). The samples were dipped in ultrapure water in a volume equal to 0.1 mL H_2O/mg f.w. and then incubated for 30 min at 40 °C ($EC_{40°}$). The samples were then reincubated at 100 °C for 10 min before EC was measured again ($EC_{100°}$). MII was calculated using the following formula:

$$MII = (EC_{40°}/EC_{100°}) * 100$$

2.7. Antioxidant Activity

2.7.1. Phenolic Compounds

Phenolic compounds were extracted from homogenized samples (*n* = 9) suspended in 3 mL of 0.1 N HCl and then incubated for three hours at 4 °C. after the incubation the samples were centrifuged for 15 min at 8000× *g*. Supernatants were collected, and the pellets were resuspended in 2 mL of 0.1 N HCl and centrifuged again for 15 min at 8000× *g*. The supernatants were pooled and brought to a final volume of 6 mL with an additional 0.1 N HCl. Total phenols amount was extrapolated owing to the protocol of Stassinos et al. [14]. The sample absorbance was measured at 724 nm. A calibration curve of chlorogenic acid (CA) (Alfa Aesar) with solutions of 10 µg/mL, 20 µg/mL, 50 µg/mL, and 100 µg/mL (y = 0.0013x − 0.0109, R^2 = 0.99) was used to calculate the concentration of phenols. The total phenolic content is expressed as µg of chlorogenic acid equivalent /g. f.w.

2.7.2. Determination of Enzymatic Activities

The enzymatic activities were determined as described by Santangeli et al. [10]. Briefly, frozen homogenized samples (200 mg f.w.), with polyvinylpolypyrrolidone (PVPP) for phenolic compounds precipitation, were suspended in 1 mL of 0.2 M sodium phosphate buffer (pH 7.0) with protease inhibitor cocktail for plant cells (Sigma). The extracts were centrifuged at 15,000× *g* at 4 °C for 30 min, and the supernatants were recovered, dialyzed overnight against distilled water to remove the excess sodium from the samples, and stored at −20 °C. The protein amount in the extracts was determined by the Bradford assay [43] and calculated using a calibration curve with bovine serum albumin (BSA) (1.25; 2.5; 5 and 10 µg/mL) (y = 0.0468x − 0.021; R^2 = 0.0998).

Superoxide dismutase (SOD) (EC 1.15.1.1) and ascorbate peroxidase (APX) (EC 1.11.1.11) activities were assayed by NPAGE (native polyacrylamide gel electrophoresis). Samples (40 µg of proteins) were loaded and separated on native polyacrylamide gels. SOD activity was visualized following the procedure described by Beauchamp and Fridovich [44]. APX activity was detected according to Mittler and Zilinskas [45]. SOD and APX activities were expressed as Arbitrary Units (A.U.), which corresponds to the pixel density of each lane obtained by the program ImageJ.

2.8. Photosynthetic Pigments and Chlorophyll Fluorescence Parameters

Homogenized samples (*n* = 9) were suspended in 3 mL of 95% ethanol at 4 °C for 1 h. in the dark. At the end of the incubation, the samples were centrifugated at 800× *g* for 10 min to remove the cell debris. Chlorophylls and carotenoid detection were performed with a spectrophotometer to measure the absorbances of the supernatants at 664 nm (chloro-

phyll a), 648.6 nm (chlorophyll b), and at 470 nm (carotenoids). Photosynthetic pigments amount was determined according to Lichtenthaler [46] and expressed as μg/mg f.w.

Imaging of chlorophyll fluorescence parameters was performed after 30 days of growth, to evaluate the activity of the photosynthetic apparatus following the protocol described by Santangeli et al. [10]. Chlorophyll fluorescence was measured with a MINI-Imaging-PAM (Walz, Germany), on the last fully expanded leaf of four representative plants per treatment. To determine F_0 (minimum fluorescence) and F_m (maximum fluorescence) leaves were maintained in the dark for 30 min. Then, leaves were adapted to a photosynthetic photon flux density (PPFD) of 55 μmoles m^{-2} s^{-1} for 10 min until the steady-state condition was reached. Maximum fluorescence ($F_{m'}$) and steady-state fluorescence (F_s), during actinic illumination, were detected through a saturation light pulse. Saturation pulse images and values of the chlorophyll fluorescence were captured. Fluorescence parameters were calculated with the following formulas:

$$F_v/F_m \text{ (Maximal quantum efficiency of PSII photochemistry)} = (F_m - F_0)/F_m$$

$$\Phi\text{PSII (Quantum efficiency of PSII photochemistry)} = (F_{m'} - F_s)/F_{m'}$$

$$\text{NPQ (Non-photochemical quenching)} = (F_m - F_{m'})/F_{m'}$$

$$\text{ETR (Electron transport rate)} = \Phi\text{PSII} * \text{PPFD} * 0.5 * \text{Abs}$$

(0.5 = light absorbed by PSII antennae; Abs = apparent absorptivity of leaf surface)

2.9. Statistical Analysis

Data are expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) was performed with the program Past 7.0. To assess the significant differences among the analyzed groups, the Tukey–Kramer method was used. All analyses were considered significant at $p \leq 0.05$ within each treatment group. When comparing inoculated groups with non-inoculated ones the significance was reported as *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

3. Results

3.1. PGPB Halotolerance

A. brasilense, B. ambifaria, H. seropedicae, and *Pseudomonas* sp. were tested for halotolerance. All strains grew at a high salt concentration (160 mM NaCl), *A. brasilense* was able to grow at 320 mM NaCl; the *Pseudomonas* sp. strain could grow at the highest salinity tested (640 mM NaCl in TSB medium) (Figure 1). The halotolerance of the *A. globiformis* strain was previously determined by Stassinos et al. [20], who reported growth of the strain in 640 mM NaCl in the TSB medium.

3.2. Plant Growth in Saline Conditions

The seed germination phase of canola is particularly sensitive to salinity; however, PGPB inoculation could help the seeds overcome this salt stress. Non-inoculated *B. napus* seeds had 96% and 82% germination rates, respectively, in non-saline and saline soils, respectively, bacterial inoculation enhanced seed germination rates up to 100% in both conditions both with and without added salt (data not shown).

The leaves of the treated and non-treated plants were not chlorotic and no necrosis was detected. In non-inoculated plants, the saline conditions reduced shoot lengths (Table 1), while the presence of bacteria significantly enhanced shoot growth in both control and salinized soils (Table 1). In addition, a significant positive effect on roots development was observed in controls inoculated with *B. ambifaria* and *A. globiformis* (Table 1). Moreover, *B. ambifaria*, *A. globiformis* and *H. seropedicae* significantly enhanced plant fresh weights in non-saline soil, and the first two species significantly increased fresh weight in saline soil (Table 1).

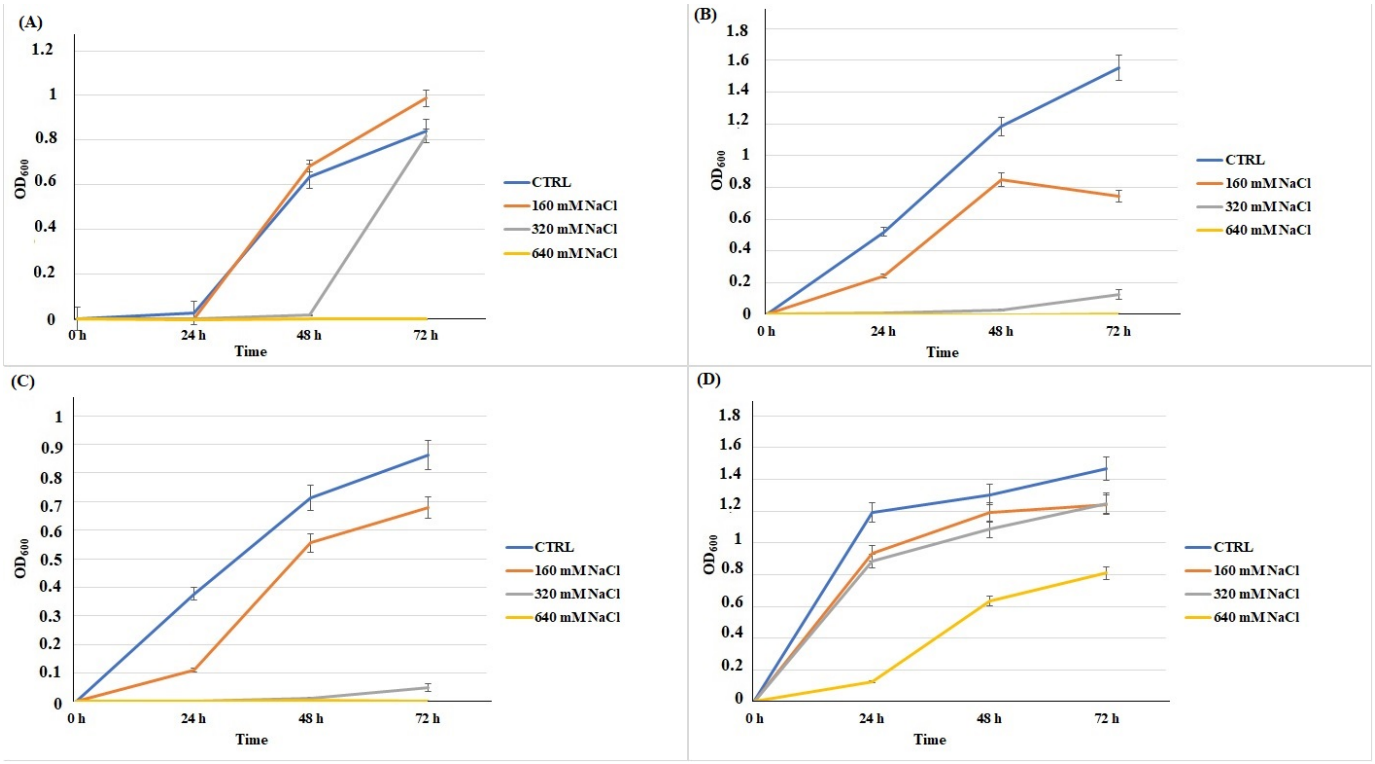

Figure 1. Growth curves of *A. brasilense* (**A**), *B. ambifaria* (**B**), *H. seropedicae* (**C**), and *Pseudomonas* sp. (**D**) in TSB media, along with different amounts of NaCl.

Table 1. Shoot length, roots length, and fresh weight of *B. napus* cv. SY Saveo grown for 30 days on non-saline soil (C) or saline soil (S). Plants were inoculated with plant growth-promoting bacteria (A = *A. brasilense*, B = *B. ambifaria*, G = *A. globiformis*, H = *H. seropedicae*, U = *Pseudomonas* sp. UW4) or not (N.I. = non-inoculated). Different letter means significant difference within the same bacterial treatment. Significant differences between inoculated or non-inoculated plants grown at the same soil condition are marked as * ($p < 0.05$), *** ($p < 0.001$) ($n = 16$).

	Shoots (cm)	Roots (cm)	Fresh Weight (g)
N.I.C.	12.68 ± 0.34 a	8.48 ± 0.37 a	1.52 ± 0.12 a
N.I.S.	10.71 ± 0.21 b	8.17 ± 0.57 a	0.95 ± 0.07 b
A.C.	15.62 ± 0.37 a ***	9.34 ± 0.49 a	1.74 ± 0.12 a
A.S.	12.87 ± 0.17 b *	6.88 ± 0.62 b	0.9 ± 0.07 b
B.C.	19.73 ± 0.17 a ***	16.92 ± 0.57 a ***	3.78 ± 0.09 a ***
B.S.	14.58 ± 0.48 b ***	8.28 ± 0.31 b	1.77 ± 0.07 b ***
G.C.	19.24 ± 0.49 a ***	18.98 ± 0.51 a ***	4.15 ± 0.17 a ***
G.S.	13.56 ± 0.31 b ***	8.77 ± 0.29 b	1.91 ± 0.07 b ***
H.C.	18.66 ± 0.55 a ***	10.35 ± 0.41 a	2.65 ± 0.08 a ***
H.S.	13.91 ± 0.62 b ***	7.64 ± 0.28 b	0.85 ± 0.08 b
U.C.	15.63 ± 0.21 a ***	8.6 ± 0.39 a	1.4 ± 0.09 a
U.S.	13.47 ± 0.24 b ***	7.41 ± 0.45 a	1.1 ± 0.08 a

3.3. Effects of PGPB on Canola Exposed to Saline Conditions

3.3.1. Water Content and Osmolyte Synthesis

Water content was reduced in plants grown in saline soil (Figure 2); nevertheless, the presence of bacteria significantly enhanced the plant water content in this condition (Figure 2). The presence of *A. globiformis* significantly ameliorated the response to salt in the water content and osmolyte synthesis (Table 2). This improvement was also detected in the absence of added salt (Table 2). The other strains did not significantly influence proline synthesis.

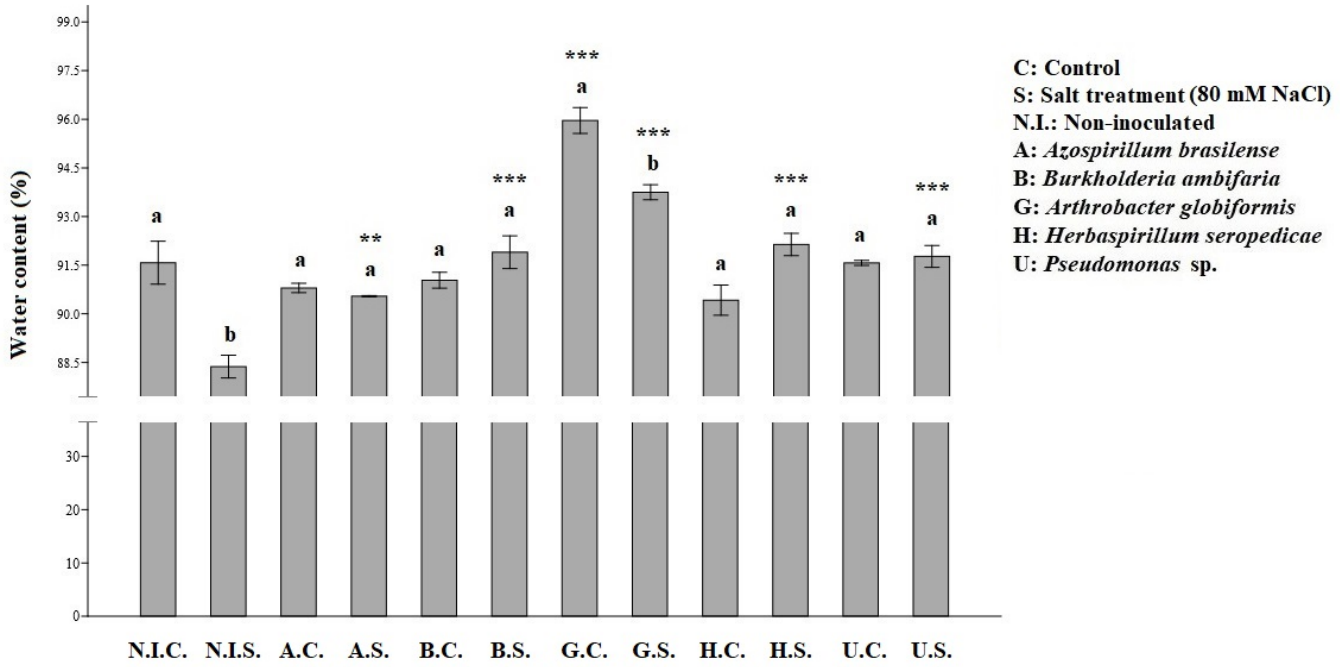

Figure 2. Water content of the plants in non-saline (C) and in saline soils (S). Data are expressed as means ± SE (n = 8). N.I. = non-inoculated. Mean values in the column marked by different letters are significantly different within the same group ($p \leq 0.05$; ANOVA and Tukey–Kramer test). Significant differences between groups are reported as ** $p < 0.01$; *** $p < 0.001$.

Table 2. Proline and phenolic compounds of *B. napus* cv. SY Saveo grown for 30 days on non-saline soil (C) or saline soil (S). Plants were inoculated with plant growth-promoting bacteria (A = *A brasilense*, B = *B ambifaria*, G = *A globiformis*, H = *H seropedicae*, U = *Pseudomonas* sp. UW4) or not (N.I. = non-inoculated). Different letter means significant difference within the same bacterial treatment. Significant differences between inoculated or not inoculated plants grown at the same soil conditions are marked as * ($p < 0.05$), *** ($p < 0.001$) (n = 9).

	Proline (nmoles/mg f.w.)	Phenolic Compounds (µg Chlorogenic Acid eq./g f.w.)
N.I.C.	2.17 ± 0.21 a	8.57 ± 0.58 a
N.I.S.	6.07 ± 0.28 b	10.46 ± 0.06 a
A.C.	1.57 ± 0.12 a	10.57 ± 0.13 a
A.S.	2.95 ± 0.17 a *	16.67 ± 0.13 b ***
B.C.	0.98 ± 0.09 a *	8.84 ± 0.18 a
B.S.	0.88 ± 0.18 a ***	9.67 ± 0.06 a
G.C.	14.62 ± 0.87 a ***	18.72 ± 0.26 a ***
G.S.	39.68 ± 2.09 b ***	20.23 ± 1.22 b ***
H.C.	0.69 ± 0.31 a *	8.50 ± 0.31 a
H.S.	0.75 ± 0.17 a ***	9.25 ± 0.07 a
U. C	1.54 ± 0.23 a	8.37 ± 0.17 a
U. S	5.85 ± 0.22 b	12.77 ± 0.31 b *

3.3.2. Membrane Injury Index (MII)

The membrane injury index (MII) is a useful biomarker for providing information about the level of damage of cell membranes; higher MII values are related to greater membrane damage. In saline soil, non-inoculated plants showed a significantly higher MII index in comparison with plants grown in control soil. However, PGPB strains significantly lowered the MII of plants exposed to salt, compared with the non-inoculated ones grown under the same conditions (Figure 3).

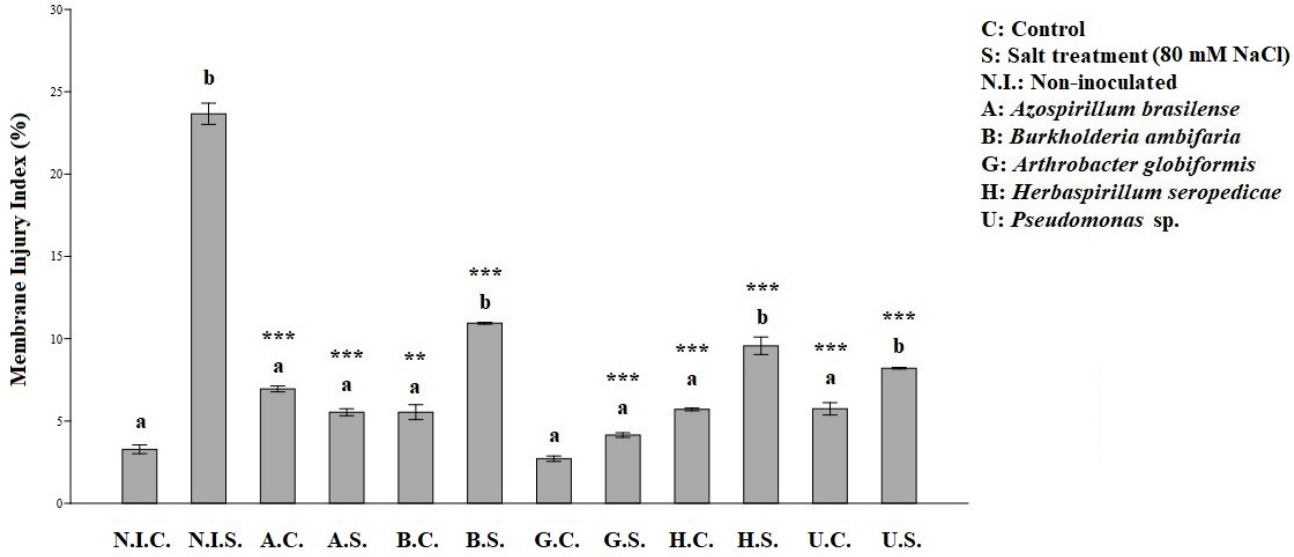

Figure 3. Membrane injury index (M.I.I.) of rapeseed grown in non-saline (C) and in saline soils (S). Data are expressed as means ± SE (n = 8). N.I. = non inoculated. Mean values in the column marked by different letters are significantly different within the same group ($p \leq 0.05$; ANOVA and Tukey–Kramer test). Significant differences between groups are reported as ** $p < 0.01$; *** $p < 0.001$.

3.3.3. Antioxidant Activity

One of the major effects of plant exposure to saline stress is the overproduction of ROS. To counteract the negative effects of ROS on cells, plants enhanced their antioxidant metabolism by producing phenolic compounds. In our experiments, a significant increase in phenolic synthesis was detected in plants inoculated with *A. globiformis* (in both soils), *A. brasilense*, and *Pseudomonas* sp. (in saline soil) (Table 2).

SOD and APX activities were evaluated to determine the enzyme-mediated antioxidant response of *B. napus*. Enhanced activity of the former was detected in plants grown in saline soil and inoculated with *A. brasilense*, *Pseudomonas*, and *A. globiformis* (Figure 4). Moreover, the effect of the latter on SOD was even evident in the non-salt exposed plants (Figure 4). APX activity was strongly enhanced in plants inoculated with *H. seropedicae* in both control and saline soils (Figure 5). No variations in the level of phenolic compounds produced and in the enzymatic activities were detected in plants inoculated with *B. ambifaria* (Table 2, Figures 4 and 5).

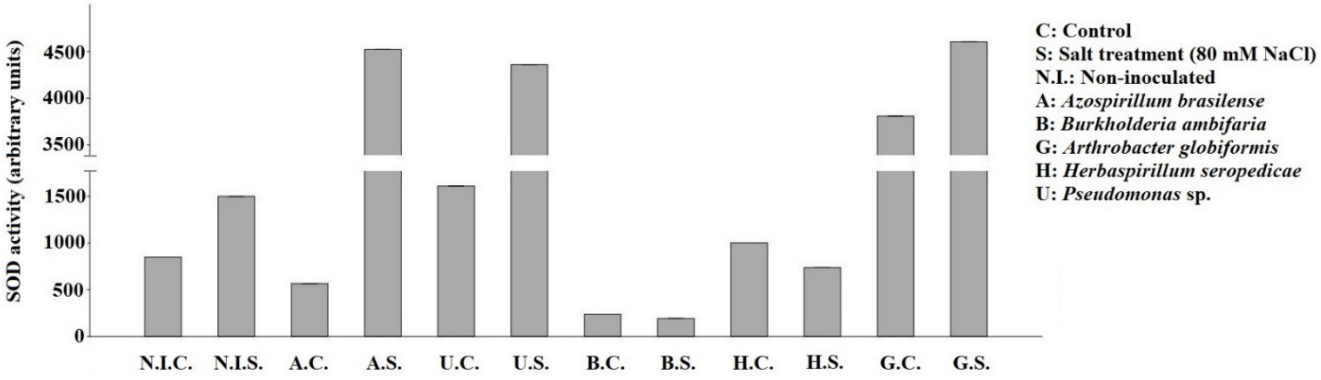

Figure 4. SOD activity of *B. napus* grown in non-saline (C) and in saline soils (S).

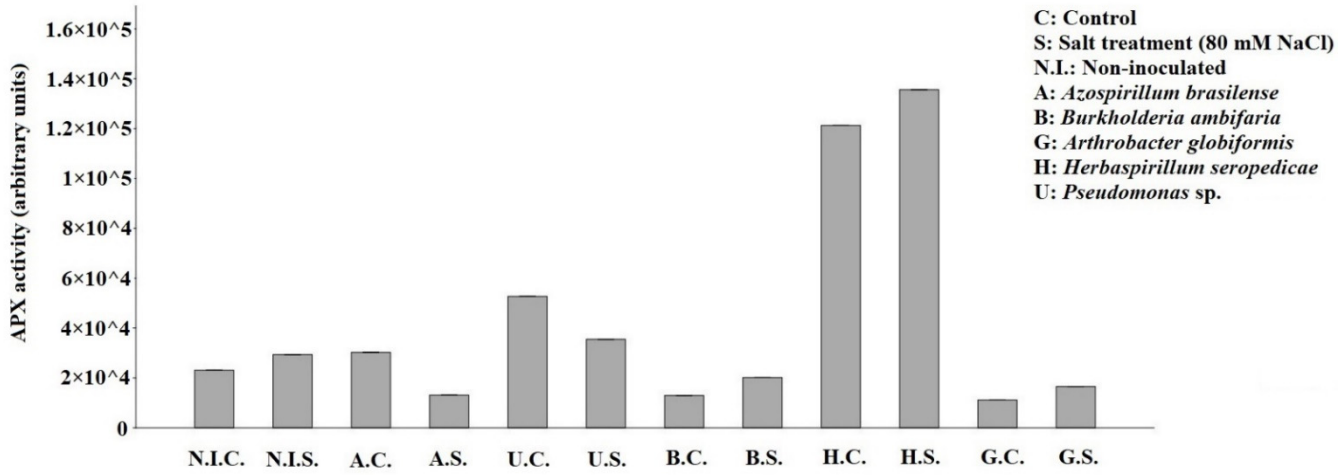

Figure 5. APX activity of *B. napus* grown in non-saline (C) and in saline soils (S).

3.3.4. Photosynthetic Pigments and Chlorophyll Fluorescence Parameters

Leaf pigments such as chlorophylls and carotenoids can also be used as indicative parameters of stress when plants are cultivated under high salt concentrations. In our experiments, a significant increase in the amount of chlorophyll was detected, under saline soil conditions, in plants inoculated with *A. brasilense*, and *Pseudomonas* sp., while plants inoculated with *B. ambifaria* showed a significant decrease in this pigment. At the same time, a significant enhancement in chlorophyll content was found under non-saline soil conditions in plants inoculated with *A. brasilense*, *A. globiformis*, and *Pseudomonas* sp., while plants inoculated with *H. seropedicae* highlighted a significant reduction in the photosynthetic pigment (Table 3). The carotenoids amount varies depending on the PGPB inoculated on canola plants. In particular, the amounts of carotenoids were significantly higher in plants grown under saline soil conditions and inoculated with *A. brasilense*, *B. ambifaria*, *H. seropedicae*, and *Pseudomonas* sp., compared with non-inoculated plants (Table 3). Moreover, a significant enhancement in carotenoid content was observed under non-saline soil conditions in plants inoculated with *A. globiformis* and *Pseudomonas* sp., (Table 3).

Table 3. Total chlorophyll and total carotenoid amounts of *B. napus* cv. SY Saveo grown for 30 days on non-saline soil (C) or saline soil (S). Plants were inoculated with plant growth-promoting bacteria (A = *A. brasilense*, B = *B. ambifaria*, G = *A. globiformis*, H = *H. seropedicae*, U = *Pseudomonas* sp. UW4) or not (N.I. = non-inoculated). Different letter means significant difference within the same bacterial treatment. Significant differences between inoculated or non-inoculated plants grown at the same soil conditions are marked as * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$) ($n = 9$).

	Total Chlorophylls (µg/g f.w.)	Total Carotenoids (µg/g f.w.)
N.I.C.	265.73 ± 5.02 a	32.13 ± 0.77 a
N.I.S.	301.54 ± 6.49 b	37.81 ± 0.79 b
A.C.	315.13 ± 17.98 a *	34.72 ± 2.1 a
A.S.	414.42 ± 22.07 b **	53.45 ± 2.31 b **
B.C.	282.33 ± 8.19 a	35.59 ± 1.5 a
B.S.	221.82 ± 15.70 b *	42.25 ± 1.31 b *
G.C.	349.58 ± 18.88 a *	43.55 ± 3.5 a *
G.S.	322.53 ± 15.01 a	41.15 ± 2.06 a
H.C.	227.82 ± 9.27 a *	30.97 ± 0.89 a
H.S.	278.47 ± 19.36 b	44.69 ± 2.96 b *
U. C	379.51 ± 49.4 a *	51.51 ± 5.7 a ***
U. S	415.59 ± 19.11 a **	51.43 ± 2.47 a **

An analysis of chlorophyll fluorescence parameters (F_v/F_m, ΦPSII, NPQ, and ETR), and their associated images were utilized to provide information on the spatial heterogeneity of leaf photosynthetic performance in plants inoculated or not with the five PGPB strains. (Figure 6, Table 4). A representative image of chlorophyll fluorescence parameters (F_v/F_m, ΦPSII, and NPQ) in a single leaf in salt-stressed or not-stressed *B. napus* plants inoculated or not with the five different PGPB is shown in Figure 6. In our experiments, a significant increase in F_v/F_m values was detected in plants inoculated with all the five PGPB strains (in saline soil) (Table 4). At the same time, a significant enhancement in ΦPSII values was found in plants inoculated with *A. brasilense*, *B. ambifaria*, *A. globiformis*, *H. seropedicae* (in saline soil). Moreover, the values of non-photochemical quenching (NPQ) showed a significant decrease in *B. napus* plants grown in non-saline soil and inoculated with PGPB (except for *A. globiformis*) in comparison with non-inoculated plants. Only *B. ambifaria* and *Pseudomonas* significantly lowered the NPQ values in plants grown in saline soil, compared with non-inoculated canola plants. Finally, the electron transport rate (ETR), which represents a proxy for photosynthesis, showed values significantly higher in plants grown under saline soil and inoculated with *A. brasilense*, *B. ambifaria*, *A. globiformis*, and *H. seropedicae* with respect to non-inoculated plants (Table 4).

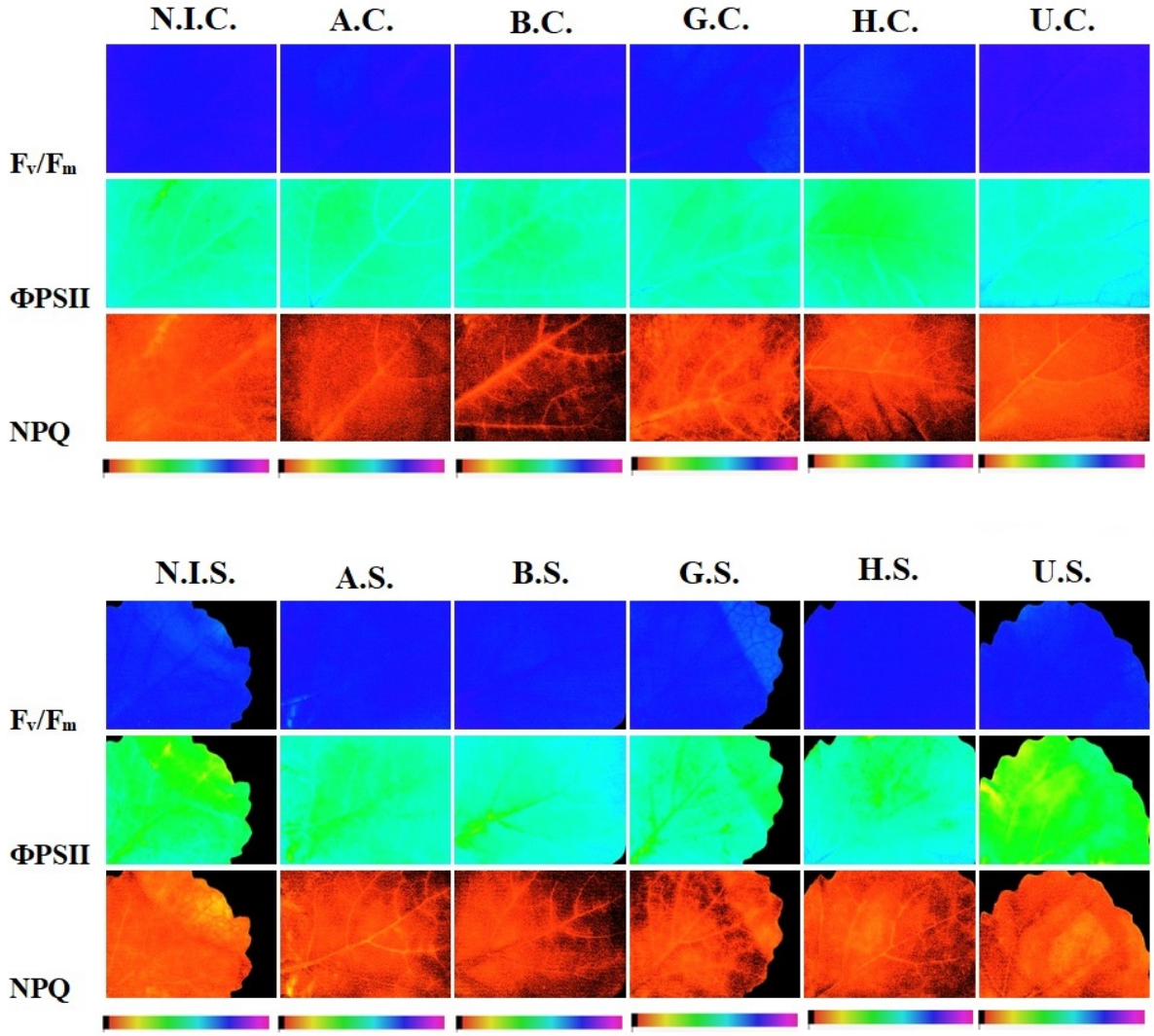

Figure 6. Chlorophyll fluorescence images of photochemistry (F_v/F_m) in a dark-adapted leaf and PSII photochemistry (ΦPSII) and non-photochemical quenching (NPQ) at steady-state with actinic illumination of 55 µmol photons m^{-2} s^{-1} measured at the end of the experiment (30th day) in *B. napus* cv. SY Saveo plants grown in non-saline (C) and in saline soils (S). The false color code depicted at the bottom of the images ranges from 0.00 (black) to 1.00. N.I. = non- inoculated; A: *A. brasilense*; B: *B. ambifaria*; G: *A. globiformis*; H: *H. seropedicae*; U: *Pseudomonas* sp.

Table 4. Chlorophyll fluorescence parameters, maximal quantum efficiency (F_v/F_m) measured in dark-adapted leaves and quantum efficiency of PSII photochemistry (ΦPSII), non-photochemical quenching (NPQ), and electron transport rate (ETR) measured at steady state with actinic light illumination of 55 µmol photons m^{-2} s^{-1} in *B. napus* cv. SY Saveo plants grown for 30 days on non-saline soil (C) or saline soil (S). Plants were inoculated with plant growth-promoting bacteria (A = *A. brasilense*, B = *B. ambifaria*, G = *A. globiformis*, H = *H. seropedicae*, U = *Pseudomonas* sp. UW4) or not (N.I. = non inoculated). Different letters mean significant differences within the same bacterial treatment. Significant differences between inoculated or non-inoculated plants grown at the same soil conditions are marked as * ($p < 0.05$) ($n = 4$).

	F_v/F_m (r.u.)	ΦPSII (r.u.)	NPQ (r.u.)	ETR (µmol electrons m^{-2} s^{-1})
N.I.C.	0.8086 ± 0.0019 a	0.488 ± 0.004 a	0.276 ± 0.015 a	11.29 ± 0.02 a
N.I.S.	0.8049 ± 0.0069 a	0.440 ± 0.004 b	0.306 ± 0.009 a	10.45 ± 0.11 b
A.C.	0.8150 ± 0.0015 a	0.499 ± 0.004 a	0.188 ± 0.009 a *	11.62 ± 0.15 a
A.S.	0.8198 ± 0.0001 b *	0.488 ± 0.004 a *	0.272 ± 0.012 b	11.30 ± 0.17 a *
B.C.	0.8195 ± 0.0010 a	0.482 ± 0.005 a	0.172 ± 0.006 a *	11.17 ± 0.08 a
B.S.	0.8241 ± 0.0009 b *	0.503 ± 0.008 a *	0.228 ± 0.020 b *	11.60 ± 0.21 a *
G.C.	0.8098 ± 0.0029 a	0.481 ± 0.016 a	0.248 ± 0.016 a	10.92 ± 0.60 a
G.S.	0.8182 ± 0.0017 a *	0.488 ± 0.003 a *	0.252 ± 0.016 a	11.35 ± 0.29 a *
H.C.	0.7979 ± 0.0080 a	0.467 ± 0.003 a	0.208 ± 0.010 a *	10.87 ± 0.07 a
H.S.	0.8186 ± 0.0014 b *	0.469 ± 0.004 a *	0.345 ± 0.028 b	10.87 ± 0.06 a *
U. C	0.8160 ± 0.0023 a	0.511 ± 0.001 a	0.164 ± 0.010 a *	11.41 ± 0.08 a
U. S	0.8200 ± 0.0016 a *	0.454 ± 0.009 b	0.239 ± 0.031 a *	10.85 ± 0.26 a

4. Discussion

Plant survival can be threatened by environmental perturbations such as climate changes and improper agronomical practices. The latter leads to loss of arable lands and other devastating consequences on crop yield. Salinization is one of the common causes of soil degradation, and since about half of the world's agricultural land is affected by salinity, the problem urgently needs a solution. According to the FAO, the level of salinization, usually reported according to the EC of the soil, can be classified into five classes, i.e., non-saline soil (EC = 0–2 dS/m), slightly saline soil (EC = 2–4 dS/m), moderately saline soil (EC = 4–8 dS/m), strongly saline soil (EC = 8–16 dS/m) and very strongly saline soil (EC > 16 dS/m). While plant salt tolerance can range from sensitive (glycophytic) to tolerant (halophytic) species.

The area dedicated to canola cultivation worldwide is growing because of its economic importance. Previous studies highlighted that, in presence of moderate saline stress (EC = 4–8 dS/m), *B. napus* growth is reduced [14,20]. To relieve the inhibitory effect of soil salinity on canola growth, different strategies have been developed and tested, including plant acclimation [10], seed priming [14], and exogenous application of methyl jasmonate [47]. In addition, the inoculation of plants with PGPB can significantly improve plant tolerance to stressful conditions [14,16]. For this study, we selected five bacterial strains based on their plant growth-promoting activities [24,29–33]. The results obtained clearly demonstrate the improvement of plant growth following inoculation with PGPB in both experimental conditions.

According to the literature [48], auxin-producing bacteria can utilize this hormone to decrease salinity-mediated osmotic stress. In this study, all of the strains tested are IAA producers [31,33,49], and this may in part explain the elevated halotolerance detected. As suggested by some authors [50], plant inoculation with halotolerant bacteria strains can represent a good tool to improve plant fitness of salt-sensitive cultivars [51].

In addition, proper plant physiological responses to stress involve eliciting different stress response mechanisms. Under this perspective, bacterial metabolic activity may play a key role [16,18,33,50], leading to better plant responses to stress. Confirming these observations, the data reported in this study showed an increase in the growth of inoculated plants grown in saline soils [20,52]. Certainly, the reduced water loss recorded in inoculated plants is one of the most important traits of the bacterial treatment of plants (Figure 2). In

inoculated and stressed plants, the higher water content could depend on the production of osmolytes, such as proline and trehalose, resulting in decreased membrane damage. In fact, plants able to accumulate proline and trehalose are better able to resist osmotic stress caused by salinity [16,18,53]. In the experiments reported here, canola inoculated with *A. globiformis* produced a very large amount of proline when grown in saline soils (Table 2), confirming the data obtained in vitro [20]. The other tested strains did not induce the overproduction of proline. Strain *Pseudomonas* sp. was previously shown to produce trehalose [18]. It is likely that the higher water content detected in inoculated and salt-exposed plants is related to a protective action of bacteria on cell membranes, as detected by a significantly lower degree of MII (Figure 3), in agreement with the data of Stassinos et al. (2021b) [20]. Such decreased membrane damage of inoculated plants can also be due to a more efficient antioxidant response, that counteracts the overproduction of reactive oxygen species (ROS) caused by salinity. ROS can cause the depolarization of cell membranes, destabilizing the membrane itself and eventually leading to cell death via cytoplasmic Ca^{2+} concentration increase [54]. However, plants with high antioxidant activity, either constitutive or induced, can withstand oxidative stress [55]. This can be achieved through the production of a plethora of antioxidant molecules, such as phenols and carotenoids [56], or by enzymatic activity [16]. The observed increase in both non-enzymatic and enzymatic antioxidant responses, induced by PGPB inoculation, confirms the literature data on the mutualism between plants and some bacterial species [20,57]. The presence of bacteria mitigates plant oxidative stress through various mechanisms consistent with the differences observed between the five PGPB strains that were tested. Plants inoculated with *A. brasilense*, *A. globiformis*, and *Pseudomonas* sp. showed an enhanced production and accumulation of phenolic compounds, compared with non-inoculated ones (Table 2). The antioxidant enzymatic activity involved SOD in plants inoculated with *A. brasilense*, *A. globiformis*, and *Pseudomonas* sp. (Figure 4) and APX activity in plants inoculated with *H. seropedicae* (Figure 5). No variations were detected in the antioxidant activity of canola inoculated with *B. ambifaria*. This bacterium may elicit other antioxidant enzymes, such as catalase, glutathione peroxidase, or guaiacol peroxidase. Therefore, further studies are needed to confirm this hypothesis.

Soil salinity can dramatically affect the development of leaves, causing chlorosis and necrosis and then early senescence, thus impacting photosynthesis as well, either directly (i.e., biosynthesis of chlorophylls, regulation of photosynthetic enzymes) or indirectly (regulation of antioxidant enzyme system) [58]. Furthermore, the induced closure of stomata reduces the rate of CO_2 assimilation [59]. Parida and Das (2005) [55] reported that the amounts of photosynthetic pigments generally decrease in salt-stressed plants, but there are also cases in which the concentration of these molecules do not vary significantly or even increase in saline conditions, as reported by Santangeli et al. [10] in canola plants. In the present study, salinity stress induced a significant increase both in contents of chlorophyll and carotenoids in non-inoculated canola plants, compared with the control, confirming findings reported by Santangeli et al. [10]. At the same time, plants inoculated with *A. brasilense* and *Pseudomonas* sp. and exposed to salt showed, compared with the controls in saline conditions, an increase in both pigments. On the contrary, plants under salt stress inoculated with *B. ambifaria* and *H. seropedicae* showed a reduction in chlorophyll content but an enhancement in carotenoids (Table 3). Our results are in accordance with previous studies that showed an increase, compared with saline control, in the amount of chlorophyll and carotenoids in plants of *Brassica napus* [52] and *Triticum durum* [50] exposed to salt stress and inoculated with PGPB strains. The increase in photosynthetic pigments in PGPB-inoculated plants suggests the capacity of bacterial inoculation to reduce the detrimental effects of salt, by ameliorating the activities of electron transporters associated with photosynthesis [60], as well as the biosynthesis of proteins and enzymes related to pigment stabilization [61]. Furthermore, Bashan et al. (2006) [62] reported that inoculation of wheat with *Azospirillum* under salt stress enhanced the production of auxiliary photoprotective pigments such as carotenoids, composed of carotenes and

xanthophylls, which may protect chlorophylls from oxidation during exposure to salt stress. As reported in the literature, the inoculation of plants with PGPB can mitigate, on a certain level, some of the deleterious effects of salinity, enhancing plant growth, biomass accumulation, yield, and photosynthetic performance [63–66]. To estimate the beneficial effects of PGPB inoculation on photosynthetic performance and to study the spatial heterogeneity of photosynthesis, chlorophyll fluorescence imaging was used. In this present study, the maximal quantum efficiency of PSII photochemistry (F_v/F_m) was not affected in salt-stressed plants, as it indicated no damage to the PSII reaction center. At the same time, canola plants inoculated with the five bacterial strains showed an increase in F_v/F_m suggesting, which indicated that maximal quantum efficiency of PSII photochemistry was enhanced by the mutualism with PGPB. F_v/F_m values reported in this study ranged from 0.797 to 0.824 (Table 4), and such values are usually measured in unstressed plants [7]. Nevertheless, as reported in the literature, the F_v/F_m ratio is considered less sensitive and responsive to the stresses in comparison with the quantum efficiency of PSII (ΦPSII) [63,67]. Salt stress can cause a decrease in ETR and ΦPSII in the photochemical quenching process, with concomitant increases in qN, NPQ, and Y(NPQ) as non-photochemical quenching mechanisms [68,69]. These results are in line with previous indications, as canola plants exposed to salt stress exhibited a significant decrease in ΦPSII and ETR and a slight but not significant increase in NPQ values with respect to the saline control plants (Table 4). On the contrary, the inoculation with the bacterial strains allowed salt-stressed plants to maintain higher ΦPSII and ETR values than non-inoculated ones, except for plants inoculated with *Pseudomonas* sp. Furthermore, the inoculation with bacterial strains reduced or did not affect the NPQ values in salt-stressed canola plants, compared with non-inoculated ones. The non-photochemical quenching (NPQ) parameter provides information about the energy dissipation by heat in the photosynthetic apparatus exerting a photoprotective action [70]. Moreover, NPQ is also involved in the protection from oxidative stress [71]. In plants exposed to salt stress conditions, the NPQ value usually increases, indicating a smaller amount of energy available for photosynthesis. Nevertheless, in the current study, the greater quantum efficiency of PSII (ΦPSII) observed in the inoculated plants under salt stress, compared with saline control plants, indicated that the reaction centers in the thylakoid membrane were open, which led to less activity of the NPQ process (lower NPQ values) [72]. In fact, salt-stressed plants inoculated with *B. ambifaria* or *Pseudomonas* sp. showed a decrease in the NPQ values, compared with saline control plants, while the inoculation with the other strains did not affect this parameter. This response allowed salt-stressed plants inoculated with PGPB to maintain an adequate balance between photosynthetic electron transport and carbon metabolism. Therefore, data of the chlorophyll fluorescence parameters analyzed in this study (F_v/F_m, ΦPSII, NPQ, and ETR) confirmed the beneficial effect of the PGPB inoculation in canola plants exposed to salt stress on the performance of the photosynthetic apparatus. Chlorophyll fluorescence images (Figure 6) showed that the parameters measured in dark-adapted leaves (F_v/F_m), especially for inoculated or non-inoculated control plants, revealed a homogeneous pattern of distribution of chlorophyll fluorescence, whereas they showed an appreciable heterogeneous pattern of light utilization and photosynthetic activity in light-adapted leaves (ΦPSII, and NPQ), especially in plants inoculated with *A. brasilense, B. ambifaria, A. globiformis* and *H. seropedicae* both in control and stress conditions. Finally, the above-mentioned enhanced antioxidant activities detected in inoculated plants avoided the detrimental effects related to a damage of photosynthetic apparatus induced by ROS and described by Gururani et al. [5]. The inoculation of canola plants with *B. ambifaria* enhanced photosynthetic activity in saline conditions even without variations in the antioxidant activity. A possible explanation for this result may be due to the production of the enzyme ACC deaminase by *B. ambifaria* [33]. It has been shown by Wang et al. [25] that the ACC deaminase containing rhizobacterium *Variovorax paradoxus* 5C-2 improved the maximal quantum energy of PSII (F_v/F_m) and the electron transport rate (ETR) of *Pisum sativum* in saline conditions. It is conceivable that this mechanism of action also occurs in plants

inoculated with *B. ambifaria*. Further studies are needed to confirm the involvement of the enzyme ACC deaminase in the protective activity of this species.

5. Conclusions

Overall, this study further elucidated the pivotal role played by halotolerant PGPB in enhancing the response of plants to salt stress. Additional insights into the multiple effects of PGPB inoculation in canola's response to salt stress were provided. These include the elicited strong and diversified antioxidant responses to overcome the oxidative stress due to ROS overproduction, as well as the synthesis of proline to counteract osmotic stress. Another beneficial effect of bacterial inoculation was the protection of photosynthetic activity, an effect that deserves further investigation. In addition to acclimation and seed priming, PGPB inoculation may represent an efficacious management strategy to be used in counteracting salt stress in crops.

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References

1. Khan, K.; Agarwal, P.; Shanware, A.; Sane, V.A. Heterologous expression of two Jatropha aquaporins imparts drought and salt tolerance and improves seed viability in transgenic *Arabidopsis thaliana*. *PLoS ONE* **2015**, *10*, e0128866. [CrossRef] [PubMed]
2. Ashraf, H.; Harris, P.J.C. Photosynthesis under stressful environments: An overview. *Photosynthetica* **2013**, *51*, 163–190. [CrossRef]
3. Hnilickova, H.; Hnilicka, F.; Martinkovà, J.; Kraus, K. Effects of salt stress on water status, photosynthesis and chlorophyll fluorescence of rocket. *Plant Soil Environ.* **2017**, *63*, 362–367. [CrossRef]
4. Sharma, A.; Kumar, V.; Shahzad, B.; Ramakrishnan, M.; Sidhu, G.P.S.; Bali, A.S.; Handa, N.; Kapoor, D.; Yadav, P.; Khanna, K.; et al. Photosynthetic response of plants under different abiotic stresses. *J. Plant Growth Reg.* **2019**, *39*, 509–531. [CrossRef]
5. Gururani, M.A.; Venkatesh, J.; Tran, L.S.P. Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Mol. Plant* **2015**, *8*, 1304–1320. [CrossRef] [PubMed]
6. Sitko, K.; Rusinowski, S.; Kalaji, H.M.; Szopinski, M.; Małkowski, E. Photosynthetic efficiency as bioindicator of environmental pressure in *A. halleri*. *Plant Physiol.* **2017**, *175*, 290–302. [CrossRef]
7. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef]
8. Pietrini, F.; Zacchini, M.; Iori, V.; Pietrosanti, L.; Ferretti, M.; Massacci, A. Spatial distribution of cadmium in leaves and its impact on photosynthesis: Examples of different strategies in willow and poplar clones. *Plant Biol.* **2010**, *12*, 355–363. [CrossRef] [PubMed]
9. Pietrini, F.; Di Baccio, D.; Iori, V.; Veliksar, S.; Lemanova, N.; Juškaitė, L.; Maruška, A.; Zacchini, M. Investigation on metal tolerance and phytoremoval activity in the poplar hybrid clone "Monviso" under Cu-spiked water: Potential use for wastewater treatment. *Sci. Total Environ.* **2017**, *592*, 412–418. [CrossRef] [PubMed]
10. Santangeli, M.; Capo, C.; Beninati, S.; Pietrini, F.; Forni, C. Gradual exposure to salinity improves tolerance to salt stress in rapeseed (*Brassica napus* L.). *Water* **2019**, *11*, 1667. [CrossRef]
11. Di Baccio, D.; Pietrini, F.; Bertolotto, P.; Pérez, S.; Barceló, D.; Zacchini, M.; Donati, E. Response of *Lemna gibba* L. to high and environmentally relevant concentrations of ibuprofen: Removal, metabolism and morpho-physiological traits for biomonitoring of emerging contaminants. *Sci. Total Environ.* **2017**, *584*, 363–373. [CrossRef] [PubMed]
12. Pietrini, F.; Passatore, L.; Fischetti, E.; Carloni, S.; Ferrario, C.; Polesello, S.; Zacchini, M. Evaluation of morpho-physiological traits and contaminant accumulation ability in *Lemna minor* L. treated with increasing perfluorooctanoic acid (PFOA) concentrations under laboratory conditions. *Sci. Total Environ.* **2019**, *695*, 133828. [CrossRef]
13. Dizge, N.; Keskinler, B. Enzymatic production of biodiesel from canola oil using immobilized lipase. *Biomass Bioenergy* **2008**, *32*, 1274–1278. [CrossRef]

14. Stassinos, P.M.; Rossi, M.; Borromeo, I.; Capo, C.; Beninati, S.; Forni, C. Enhancement of *Brassica napus* tolerance to high saline conditions by seed priming. *Plants* **2021**, *10*, 403. [CrossRef]
15. Cheng, Z.; Park, E.; Glick, B.R. 1-Aminocyclopropane-1-carboxylate (ACC) deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Can. J. Microbiol.* **2007**, *53*, 912–918. [CrossRef]
16. Forni, C.; Duca, D.; Glick, B.R. Mechanism of plant response to salt and drought stress and their alteration by rhizobacteria. *Plant Soil* **2017**, *410*, 335–356. [CrossRef]
17. Mayak, S.; Tirosh, T.; Glick, B.R. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant. Physiol. Biochem.* **2004**, *42*, 565–572. [CrossRef] [PubMed]
18. Orozco-Mosqueda, M.C.; Duan, J.; Di Bernardo, M.; Zetter, E.; Campos-García, J.; Glick, B.R.; Santoyo, G. The production of ACC deaminase and trehalose by the plant growth promoting bacterium *Pseudomonas* sp. UW4 synergistically protect tomato plants against salt stress. *Front. Microbiol.* **2019**, *10*, 1392. [CrossRef]
19. Siddikee, M.A.; Glick, B.R.; Chauhan, P.S.; Yim, W.J.; Sa, T. Enhancement of growth and salt tolerance of red pepper seedlings (*Capsicum annuum* L.) by regulating stress ethylene synthesis with halotolerant bacteria containing ACC deaminase activity. *Plant Physiol. Biochem.* **2011**, *49*, 427–434. [CrossRef]
20. Stassinos, P.M.; Rossi, M.; Borromeo, I.; Capo, C.; Beninati, S.; Forni, C. Amelioration of salt stress tolerance in rapeseed (*Brassica napus*) cultivars by seed inoculation with *Arthrobacter globiformis*. *Plant Biosyst.* **2021**, 1–14. [CrossRef]
21. Glick, B.R. Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica* **2012**, *2012*, 963401. [CrossRef] [PubMed]
22. Brock, A.K.; Berger, B.; Mewis, I.; Ruppel, S. Impact of the PGPB *Enterobacter radicincitans* DSM 16656 on growth, glucosinolate profile, and immune responses of *Arabidopsis thaliana*. *Microb. Ecol.* **2012**, *65*, 661–670. [CrossRef]
23. Hussein, A.H.; Joo, J.H. Plant growth-promoting rhizobacteria improved salinity tolerance of *Lactuca sativa* and *Raphanus sativus*. *J. Microbiol. Biotechnol.* **2018**, *28*, 938–945. [CrossRef] [PubMed]
24. Penrose, D.M.; Glick, B.R. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant* **2003**, *118*, 10–15. [CrossRef] [PubMed]
25. Wang, Q.; Dodd, I.C.; Belimov, A.A.; Jiang, F. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase growth and photosynthesis of pea plants under salt stress by limiting Na^+ accumulation. *Funct. Plant Biol.* **2016**, *43*, 161–172. [CrossRef]
26. Glick, B.R.; Gamalero, E. Recent development in the study of plant microbiomes. *Microorganisms* **2021**, *9*, 1533. [CrossRef] [PubMed]
27. Yaghoubi Khanghahi, M.; Strafella, S.; Crecchio, C. Changes in photo-protective energy dissipation of photosystem II in response to beneficial bacteria consortium in durum wheat under drought and salinity stresses. *Appl. Sci.* **2020**, *10*, 5031. [CrossRef]
28. Yaghoubi Khanghahi, M.; Leoni, B.; Crecchio, C. Photosynthetic responses of durum wheat to chemical/microbiological fertilization management under salt and drought stresses. *Acta. Physiol. Plant* **2021**, *43*, 123. [CrossRef]
29. Balsanelli, E.; De Baura, V.A.; Pedrosa, F.D.O.; Souza, M.d.S.; Monteiro, R.A. Exopolysaccharide biosynthesis enables mature biofilm formation on abiotic surfaces by *Herbaspirillum seropedicae*. *PLoS ONE* **2014**, *9*, e110392. [CrossRef] [PubMed]
30. Del Gallo, M.; Haegi, A. Characterization and quantification of exocellular polysaccharides in *Azospirillum brasilense* and *Azospirillum lipoferum*. *Symbiosis* **1990**, *9*, 155–161.
31. Forni, C.; Haegi, A.; Del Gallo, M.; Grilli Caiola, M. Production of polysaccharides by *Arthrobacter globiformis* associated with *Anabaena azollae* in *Azolla* leaf cavity. *FEMS Microb. Lett.* **1992**, *93*, 269–274. [CrossRef]
32. Forni, C.; Riov, J.; Grilli Caiola, M.; Tel-Or, E. Indole-3-acetic acid (IAA) production by *Arthrobacter* species isolated from *Azolla*. *J. Gen. Microbiol.* **1992**, *138*, 377–381. [CrossRef]
33. Pellegrini, M.; Pagnani, G.; Rossi, M.; D'Egidio, S.; Del Gallo, M.; Forni, C. *Daucus carota* L. seed inoculation with a consortium of bacteria improves plant growth, soil fertility status and microbial community. *Appl. Sci.* **2021**, *11*, 3274. [CrossRef]
34. Gamalero, E.; Berta, G.; Massa, N.; Glick, B.R.; Lingua, G. Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences for the growth of cucumber under salt-stress conditions. *J. Appl. Microbiol.* **2009**, *108*, 236–245. [CrossRef] [PubMed]
35. Nascimento, F.X.; Vicente, C.S.L.; Barbosa, P.; Espada, M.; Glick, B.R.; Mota, M.; Oliveira, S. Evidence for the involvement of ACC deaminase from *Pseudomonas putida* UW4 in the biocontrol of pine wilt disease caused by *Bursaphelenchus xylophilus*. *BioControl* **2012**, *58*, 427–433. [CrossRef]
36. Onofre-Lemus, J.; Hernandez-Lucas, I.; Girard, L.; Caballero-Mellado, J. ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species, and its growth promoting effect on tomato plants. *Appl. Environ. Microbiol.* **2009**, *75*, 6581–6590. [CrossRef] [PubMed]
37. Nascimento, F.X.; Rossi, M.J.; Soares, C.R.F.S.; McConkey, B.J.; Glick, B.R. New insights into ACC deaminase phylogeny, evolution and evolutionary significance. *PLoS ONE* **2014**, *9*, e99168. [CrossRef]
38. Nordstedt, N.P.; Jones, M.L. Isolation of rhizosphere bacteria that improve quality and water stress tolerance in greenhouse ornamentals. *Front. Plant Sci.* **2020**, *11*, 826. [CrossRef] [PubMed]
39. Siddikee, A.; Chauhan, P.S.; Anandham, R.; Han, G.H.; Sa, T. Isolation, Characterization, and Use for Plant Growth Promotion under Salt Stress, of ACC Deaminase-Producing Halotolerant Bacteria Derived from Coastal Soil. *J. Microbiol. Biotechnol.* **2010**, *27*, 1724. [CrossRef] [PubMed]

40. Duan, J.; Jiang, W.; Cheng, Z.; Heikkila, J.J.; Glick, B.R. The complete genome sequence of the plant growth-promoting bacterium *Pseudomonas putida* UW4. *PLoS ONE* **2013**, *8*, e58640. [CrossRef]
41. Hoagland, D.R.; Arnon, D.I. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stat. Circ.* **1950**, *347*, 1–32.
42. Zeng, F.; Shabala, L.; Zhou, M.; Zhang, G.; Shabala, S. Barley responses to combined waterlogging and salinity stress: Separating effects of oxygen deprivation and elemental toxicity. *Front. Plant Sci.* **2013**, *4*, 313. [CrossRef] [PubMed]
43. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
44. Beauchamp, C.; Fridovich, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* **1971**, *44*, 276–287. [CrossRef]
45. Mittler, R.; Zilinskas, B.A. Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate-dependent reduction of nitroblue tetrazolium. *Anal. Biochem.* **1993**, *212*, 540–546. [CrossRef] [PubMed]
46. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigment of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382. [CrossRef]
47. Ahmadi, F.I.; Karimi, K.; Struik, P.C. Effect of exogenous application of methyl jasmonate on physiological and biochemical characteristics of *Brassica napus* L. cv. Talaye under salinity stress. *S. Afr. J. Bot.* **2018**, *115*, 5–11. [CrossRef]
48. Bianco, C.; Imperlini, E.; Calogero, R.; Senatore, B.; Amoresano, A.; Carpentieri, A.; Pucci, P.; Defez, R. Indole-3-acetic acid improves *Escherichia coli*'s defences to stress. *Arch. Microbiol.* **2006**, *185*, 373–382. [CrossRef]
49. Patten, C.L.; Glick, B.R. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl. Environ. Microbiol.* **2002**, *68*, 3795–3801. [CrossRef] [PubMed]
50. Djebaili, R.; Pellegrini, M.; Rossi, M.; Forni, C.; Smati, M.; Del Gallo, M.; Kitouni, M. Characterization of plant growth-promoting traits and inoculation effects on *Triticum durum* of actinomycetes isolates under salt stress conditions. *Soil Syst.* **2021**, *5*, 26. [CrossRef]
51. Etesami, H.; Glick, B.R. Halotolerant-plant growth-promoting bacteria: Prospects for mitigating salinity stress in plants. *Environ. Exptl. Bot.* **2020**, *178*, 104124. [CrossRef]
52. Abdel Latef, A.A.H.; Omer, A.M.; Badawy, A.A.; Osman, M.S.; Ragaey, M.M. Strategy of salt tolerance and interactive impact of *Azotobacter chroococcum* and/or *Alcaligenes faecalis* inoculation on canola (*Brassica napus* L.) plants grown in saline soil. *Plants* **2021**, *10*, 110. [CrossRef] [PubMed]
53. Yoshiba, Y.; Kiyosue, T.; Nakashima, K.; Yamaguchi-Shinozaki, K.; Shinozaki, K. Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiol.* **1997**, *38*, 1095–1102. [CrossRef] [PubMed]
54. Stark, G. Functional consequences of oxidative membrane damage. *J. Membr. Biol.* **2015**, *205*, 1–16. [CrossRef] [PubMed]
55. Parida, A.K.; Das, A.B. Salt tolerance and salinty effects on plants: A review. *Ecotoxicol. Environ. Saf.* **2005**, *60*, 324–349. [CrossRef] [PubMed]
56. Abogadallah, G.M. Antioxidative defense under salt stress. *Plant Signal. Behav.* **2010**, *5*, 369–374. [CrossRef] [PubMed]
57. Mahmood, A.; Can Turgay, O.; Farooq, M.; Hayat, R. Seed biopriming with plant growth promoting rhizobacteria: A review. *FEMS Microbiol. Ecol.* **2016**, *92*, 1–14. [CrossRef] [PubMed]
58. Yang, Z.; Li, J.-L.; Liu, L.-N.; Xie, Q.; Sui, N. Photosynthetic regulation under salt stress and salt-tolerance mechanism of sweet sorghum. *Front. Plant Sci.* **2020**, *10*, 1722. [CrossRef]
59. Flexas, J.; Diaz-Espejo, A.; Galmés, J.; Kaldenhoff, R.; Medrano, H.; Ribas-Carbo, M. Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. *Plant Cell Environ.* **2007**, *30*, 1284–1298. [CrossRef]
60. Pinnola, A.; Staleva-Musto, H.; Capaldi, S.; Ballottari, M.; Bassi, R. Electron transfer between carotenoid and chlorophyll contributes to quenching in the LHCSR1 protein from *Physcomitrella patens*. *Biochim. Biophys. Acta-Bioenerg.* **2016**, *1857*, 1870–1878. [CrossRef]
61. Enebe, M.C.; Babalola, O.O. The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: A survival strategy. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 7821–7835. [CrossRef]
62. Bashan, Y.; Bustillos, J.J.; Leyva, L.A.; Hernandez, J.P.; Bacilio, M. Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by *Azospirillum brasilense*. *Biol. Fertil. Soils* **2006**, *42*, 279–285. [CrossRef]
63. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef] [PubMed]
64. Etesami, H. Enhanced phosphorus fertilizer use efficiency with microorganisms. In *Nutrient Dynamics for Sustainable Crop Production*; Meena, R., Ed.; Springer: Singapore, 2020; pp. 215–245. [CrossRef]
65. Mokrani, S.; Nabti, E.H.; Cruz, C. Current advances in plant growth promoting bacteria alleviating salt stress for sustainable agriculture. *Appl. Sci.* **2020**, *10*, 7025. [CrossRef]
66. Shilev, S. Plant-growth-promoting bacteria mitigating soil salinity stress in plants. *Appl. Sci.* **2020**, *10*, 7326. [CrossRef]
67. Malinská, H.; Pidlisnyuk, V.; Nebeská, D.; Erol, A.; Medžová, A.; Trögl, J. Physiological response of *Miscanthus* x *giganteus* to plant growth regulators in nutritionally poor soil. *Plants* **2020**, *9*, 194. [CrossRef] [PubMed]
68. Acosta-Motos, J.R.; Díaz-Vivancos, P.; Álvarez, S.; Fernández-García, N.; Sánchez-Blanco, M.J.; Hernández, J.A. NaCl-induced physiological and biochemical adaptative mechanism in the ornamental *Myrtus cummunis* L. plants. *J. Plant Physiol.* **2015**, *183*, 41–51. [CrossRef] [PubMed]

69. Ikbal, F.; Hernández, J.A.; Barba-Espín, G.; Koussa, T.; Aziz, A.; Faize, M.; Diaz-Vivancos, P. Enhanced salt-induced antioxidative responses involve a contribution of polyamine biosynthesis in grapevine plants. *J. Plant Physiol.* **2014**, *171*, 779–788. [CrossRef] [PubMed]
70. Lambrev, P.H.; Miloslavina, Y.; Jahns, P.; Holzwarth, A.R. On the relationship between non-photochemical quenching and photoprotection of photosystem II. *Biochem. Biophys. Acta* **2012**, *1817*, 760–769. [CrossRef] [PubMed]
71. Azzabi, G.; Pinnola, A.; Betterle, N.; Bassi, R.; Alboresi, A. Enhancement of non-photochemical quencing in the Bryophyte *Physcomitrella patens* during acclimation to salt and osmotic stress. *Plant Cell Physiol.* **2012**, *53*, 1815–1825. [CrossRef] [PubMed]
72. Van Amerongen, H.; Chmeliov, J. Instantaneous switching between different modes of non-photochemical quenching in plants. Consequences for increasing biomass production. *Biochim. Biophys. Acta-Bioenerg.* **2020**, *1861*, 148119. [CrossRef] [PubMed]