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Effect of *Bacillus* spp. and *Brevibacillus* sp. on the Photosynthesis and Redox Status of *Solanum lycopersicum*

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Abstract: Plant-growth-promoting bacteria (PGPB) are gaining attention as a sustainable alternative to current agrochemicals. This study evaluated the impact of three *Bacillus* spp. (5PB1, 1PB1, FV46) and one *Brevibacillus* sp. (C9F) on the important crop tomato (*Solanum lycopersicum*) using the model cv. 'MicroTom'. The effects of these isolates were assessed on (a) seedlings' growth and vigor, and (b) adult potted plants. In potted plants, several photosynthetic parameters (chlorophylls (*a* and *b*), carotenoids and anthocyanins contents, transpiration rate, stomatal conductance, net CO_2 photosynthetic rate, and intercellular CO_2 concentration, and on chlorophyll fluorescence yields of light- and dark-adapted leaves)), as well as soluble sugars and starch contents, were quantified. Additionally, the effects on redox status were evaluated. While the growth of seedlings was, overall, not influenced by the strains, some effects were observed on adult plants. The *Bacillus safensis* FV46 stimulated the content of pigments, compared to C9F. *Bacillus zhangzhouensis* 5PB1 increased starch levels and was positively correlated with some parameters of the photophosphorylation and the gas exchange phases. Interestingly, *Bacillus megaterium* 1PB1 decreased superoxide (O_2^-) content, and *B. safensis* FV46 promoted non-enzymatic antioxidant defenses, increasing total phenol content levels. These results, conducted on a model cultivar, support the theory that these isolates differently act on tomato plant physiology, and that their activity depends on the age of the plant, and may differently influence photosynthesis. It would now be interesting to analyze the influence of these bacteria using commercial cultivars.

Keywords: biofertilizers; plant-growth-promoting bacteria; sustainable agriculture; *Solanum lycopersicum*; chlorophyll a fluorescence; gas exchange

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1. Introduction

Increasing global food demand has made agricultural practices extensively dependent on chemical fertilizers and pesticides. These agrochemicals are of extreme importance to food security, but contaminate the environment (e.g., water pollution and soil degradation), affecting entire ecosystems and human health [1,2]. The sustainability of food production became a priority, increasing the interest in new environmentally friendly tools to replace synthetic agrochemicals or, at least, reduce their impact [3]. One of the proposed alternatives to traditional agrochemicals is the use of plant-growth-promoting bacteria (PGPB) [4,5].

PGPB can improve plant growth and health in several ways. Direct benefits affect the plant metabolism, including the levels of phytohormone-like indole acetic acid

(IAA) [6,7], and improve the availability of nutrients through, for example, the production of siderophores [8,9], nitrogen fixation [10,11], or phosphate solubilization [12,13]. Indirect benefits include protection against pathogens through the production of antibiotic compounds [14,15], direct competition for nutrients [9,16], or by inducing Systemic Resistance in plants [17]. PGPB can also increase plants' tolerance to adverse environmental conditions (e.g., high salinity and drought), by regulating hormone-synthetic pathways; for example, by influencing the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase [18], which reduces stress ethylene production [19].

PGPB-mediated changes in phytohormone levels, and improved nutrition, ultimately lead to metabolic changes. Several studies suggest that PGPB inoculation might benefit the photosynthetic metabolism in many plant species. *Bacillus amyloliquefaciens* and the mycorrhizal fungi *Rhizophagus irregularis* improved the photosynthetic efficiency of *Cyrtostylis rotundifolia*, *Thymus serpyllum*, *Fragaria vesca*, and *Trifolium repens* [20]. Another study in sugar beet plants (*Beta vulgaris*) showed that inoculation with strains of three PGPB species (*Bacillus pumilus*, *Chryseobacterium indologene,* and *Acinetobacter johnsonii*) increased dry weight and plant height, and stimulated the maximum efficiency (F_v/F_m) of Photosystem II (PSII), carbon assimilation, and chlorophyll content (Chl), which augmented carbohydrates' synthesis [21].

PGPB may increase plants' resilience and response to stressful conditions. Stefan et al. [22] showed that the inoculation of runner bean plants (*Phaseolus coccineus*) with two siderophores and IAA-producing PGPB strains augmented chlorophyll content and photosynthetic rates, and improved water-use efficiency compared to control plants. The inoculation of drought-stressed cucumber plants with a combination of three PGPB strains, *Bacillus cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21, increased the leaf chlorophylls contents compared to control plants, even under adverse water conditions [23]. The halotolerant PGPB-strain *Curtobacterium albidum* SRV4 increased Chl *a* and *b* and carotenoid contents in *Oryza sativa* plants grown under salt stress, compared to uninoculated control plants [24]. Furthermore, the inoculation of *Pseudomonas aeruginosa* and *Burkholderia gladioli* on cadmium-stressed tomato plants improved gas-exchange parameters such as net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate, compared to control plants. Besides that, an increase in the levels of the photosynthetic pigments, as well as an upregulation of carbohydrate content, were observed in PGPB-treated plants [25]. *B. subtilis* and *P. fluorescens* inoculation of radish plants stimulated plant growth, alone or in combination with saline stress, and stimulated Chl *a*, Chl *b*, Chl *a*/*b* ratio and carotenoids contents in leaves. Similarly, inoculation with a *Bacillus* sp. in lettuce plants under saline stress conditions increased Chl content, the photosynthetic rate, and stomatal conductance [26].

Tomato (*S. lycopersicum*) is one of the most important crops in the world, with more than 180 million tons of tomatoes being produced globally in 2018 (FAOSTAT, http://www.fao.org/faostat/ (accessed on 2 February 2021)). Their nutritional properties (e.g., highly rich in vitamin A and C), and versatility of processing into multiple products make tomatoes vegetables of extreme value. Its production has increased in recent years, with a trend towards future increase. It is highly important to ensure the dual compromise of food security and safety by increasing tomatoes' yield and quality without compromising the environment. Local PGBP isolates represent a sustainable solution, but the efficacy of regional PGPB strains on crops, and the potential to select the most adequate strains according to the plant's age and traits to improve, is in its infancy.

This study aimed to evaluate the physiological effects of four different isolates of species often associated with PGPB, recently collected in Portugal. This work is focused on comparing the efficiency of these candidate PGPB strains on the important crop tomato, to see if they differently influence (a) seedlings' vigor and biomass production (fresh and dry weight), and (b) the physiological parameters of mature plants, regarding photosynthesis and redox status.

2. Materials and Methods

2.1. Bacterial Isolates and Growth

The four selected isolates belong to the bacterial collections of the University of Porto/Instituto Politécnico de Viana de Castelo, and University of Trás os Montes e Alto Douro (Portugal). The selected strains were isolated between 2017 and 2018 in the North of Portugal, from chestnut roots (C9F, 1PB1, and 5PB1) and kiwifruit phyllosphere (FV46). The isolates were identified through 16S rRNA gene sequencing and phylogenetic analysis as *Bacillus megaterium* (1PB1), *Bacillus zhangzhouensis* (5PB1), *Bacillus safensis* (FV46), and *Brevibacillus lateosporus* (C9F). GenBank accession numbers for the 16S rRNA sequence are respectively MW160938, MW160944, MW160957, and MW160949. All strains were maintained at −80 °C. Before the inoculation, bacterial isolates were grown in Luria-Bertani (LB) broth medium (Liofilchem, Italy), and incubated at 25 °C ± 1 °C, at 160 rpm. For inoculation studies, cultures were adjusted to an optical density of λ = 600 (OD600) at 0.100 ± 0.005.

2.2. Seed Biopriming and Plant Growth

Seeds of tomato cv. MicroTom (Moles Seeds Ltd., Colchester, United Kingdom) were randomly chosen and disinfected in commercial bleach (5% OCl^-) at a final concentration of 50%, washed, and soaked for 1 h in sterile water. To assess if the strains had beneficial effects on tomatoes, two approaches were performed, one using seedlings, and another using adult potted plants.

Effects of the strains on tomato seedlings growth and vigor: ten randomly selected soaked seeds were placed in the middle line of each petri dish (11 × 11 cm) with Hoagland medium (pH = 5.7) with 0.7% agar (*w*/*v*). On day 3, the above-mentioned bacterial cultures were inoculated in a line below (0.5 cm) each seed, using a sterile 1 µL loop. Plate dishes were disposed vertically on a phytotron under a 16h light photoperiod, 200 µmol m^{-2} s^{-1} of PAR radiation (white fluorescent lamps OSRAM, Germany), at 25 °C ± 1 °C. After 12 days of treatment, seedlings' shoot and root length, as well as biomass, were measured. Two independent experiments with similar conditions were established with a one-week interval.

Effects of the strains on tomato plant photosynthesis and redox status: soaked seeds were inoculated and incubated as above (but the agar was replaced by filter paper). Six days after germination, inoculated seedlings were transferred to 0.9 L pots with peat: perlite and watered with $\frac{1}{2}$ strength Hoagland solution (pH = 5.7), two times a week, to 100% field capacity. Plant growth took place under greenhouse conditions, between March and July, with mean and maximum air temperatures ranging from 13 and 18 °C (March) to 25 and 33 °C (July), respectively [27,28]. Photoperiod ranged from 12 h of light (March) to 15 h of light (July). After 121 days of inoculation (i.e., 130-day-old plants), all plants were synchronized in the flowering/fruit production stage. At this period, chlorophyll *a* fluorescence and gas-exchange measurements took place. Collected leaves were immediately frozen in liquid nitrogen, and stored at −80 °C for further quantification of total soluble sugars, starch, pigments, total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, and superoxide. Two independent experiments with similar conditions were established.

2.3. Pigments Quantification

Pigment quantification was performed according to Sims and Gamon [29], using three pool replicates (each consisting of two top mature leaves from each plant; eight plants were used per pool). Leaves were powdered in N_2, and stored at −75° until further use. Frozen leaf-powder (100 mg) of each were macerated (~30 s) with 1 mL of extraction Acetone: Tris-HCl buffer on ice. After adding another 0.5 mL of the extraction buffer, tubes were vortexed and centrifuged (10 min) at 10,000× *g* at 4 °C. Supernatants were transferred to falcon tubes, and pellets were mixed with the extraction buffer (1.5 mL), vortexed, and centrifuged again. Supernatants were added to previous ones. Absorbances were read (in triplicate) at 663, 537, 647, and 470 nm using the Multiskan™ GO Microplate

Spectrophotometer (Thermo Scientific™, USA). Chlorophyll *a*, chlorophyll *b*, carotenoid, and anthocyanin contents (mol/g) were estimated according to Sims and Gamon [29].

2.4. Carbohydrates Content (TSS and Starch)

Total soluble sugars (TSS) content was quantified by the anthrone method [30], and the starch content according to Osaki et al. [31], using three pool replicates (each pool consisting of two top mature leaves from each plant; eight plants were used per pool). For TSS determination, 50 mg of frozen samples were macerated with 1.5 mL 80% ethanol, and 80% ethanol was added to a final volume of 10 mL. After incubation at 80 °C for 1 h, tubes were kept in ice (10 min), and centrifuged (10 min) at 10,000× *g*, at 4 °C. Supernatants were transferred to new tubes and the pellets were preserved. For the anthrone reaction, 30 µL of each supernatant was mixed with 750 µL of the anthrone solution (40 mg anthrone in 1:20 *v/v* (H_2O: H_2SO_4)). Reaction tubes were incubated (10 min) at 100 °C and cooled in ice (15 min). Absorbances were read at 625 nm using the Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific™, USA). A standard curve for glucose was used.

For starch content quantification, pellets reserved from the TSS quantification protocol were mixed with 5mL of 30% perchloric acid, and vortexed. Tubes were incubated for 1 h at 60 °C, and then kept in ice for 15 min, vortexed, and centrifuged (10 min) at 10,000× *g*, at 4 °C. Each supernatant (30 µL) was mixed with 750 µL of anthrone solution. Reaction tubes were incubated at 100 °C (10 min), and vortexed. The same process for TSS quantification was used here, and the content of starch was determined using a standard curve for glucose.

2.5. Photosynthetic Performance and Gas Exchange

Top mature leaves of eight plants of each treatment were used for gas exchange and chlorophyll *a* fluorescence determination. The LI-6400XT Photosynthesis System Li-COR (Lincoln, Nebraska, USA) was used for the analysis of gas exchanges. Transpiration rate (E, mol m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}), net CO_2 photosynthetic rate (P_N, µmol m^{-2} s^{-1}), and intercellular CO_2 concentration (Ci, ppm) were determined according to Dias et al. [32]. Chlorophyll *a* fluorescence was determined by measuring the minimal fluorescence yield (F0) in leaves adapted to dark for 40 min, using the LI-6400XT Photosynthesis System (Li-COR, USA) fluorometer. Following adaptation to dark, a 0.7 s saturating light pulse (>1500 µmol m^{-2} s^{-1}) was applied, and the maximum fluorescence yield was determined (F_m). F'_0 and F'_m were also determined in leaves adapted to light for 30 min. Variable fluorescence values $F_v = F_m - F_0$ and $F'_v = F'_m - F'_0$ were estimated to assess the maximum efficiency of PSII and the effective photochemical efficiency of PSII (Φ_{PSII}). Additionally, the photochemical quenching ($qP = (F'_m - F')/(F'_m - F'_0)$), non-photochemical quenching ($NPQ = (F_m - F'_m)/F'_m$) and effective efficiency of PSII ($\Phi_{PSII} = (F'_m - F')/F'_m$) were determined [33,34].

2.6. Total Phenolic Content

Total phenolic content was quantified following the Folin–Ciocalteau method, according to Dewanto et al. [35]. Samples of frozen top mature leaves (100 mg, in triplicates) of each condition were macerated with 2 mL of extraction buffer [H_2O: methanol (9:1)] at −4 °C. Filtered extracts were collected in 15 mL falcon tubes, and centrifuged at 2500 rpm (10 min) and 4 °C. Each extract (125 µL), and 125 µL of gallic acid standards (0, 25, 50, 100, 150, 200, 250, and 500 µg/mL) were transferred to new tubes, to which 500 µL H_2O and 125 µL of the Folin–Ciocalteau reagent were added. After incubation (6 min) in the dark, 1.25 mL of 7% Na_2CO_3 and 1 mL of water were added. Tubes were incubated (90 min), and absorbances were read at 760 nm using the Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

2.7. DPPH Scavenging Activity

The scavenging activity of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was determined according to Harkat-Madouri et al. [36] with some modifications. Frozen leaves (100 mg,

in triplicates) of each condition were used. Top mature leaves were macerated with 3 mL of methanol at 4 °C. Extracts were vacuum-filtered and the filtered extracts were collected in 15 mL falcon tubes and centrifuged at 2500 rpm (10 min). Each sample (150 µL) was diluted with 100 µL of methanol and 1.25 mL of DPPH 0.1 mM was added. Tubes were incubated for 30 min. in the dark. Absorbances were read at 517 nm using the Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer. For the antiradical capacity, a standard curve of gallic acid following the concentrations 0, 5, 10, 15, 20, 25, 30, 35, and 40 µg mL^{-1} was used.

2.8. Superoxide (O_2^-)

Superoxide (O_2^-) was measured using the Nitroblue tetrazolium (NBT), according to Gajewska and Sklodowska [37]. Briefly, 100 mg of frozen leaves was used (in triplicate). Samples were mixed with the extraction buffer (2 mL), containing 0.01 M phosphate buffer (pH = 7.8), 0.05% NBT diluted in 100 µL DMSO, and 10 mM sodium azide, incubated (1h) at room temperature, and centrifuged at 13,000× *g* for 2 min, at 4 °C. Samples were then incubated at 85 °C (15 min) and kept in ice (10 min). Absorbances were read at 580 nm using the Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

2.9. Statistical Analysis

Experiments regarding the impacts of the strains on seedlings were repeated twice. In each experiment, 20 seedlings were used per condition for the shoot and root length and weight measurements. Experiments regarding the effects of the strains on mature potted plants were repeated twice. In each experiment, 10 mature plants were grown per condition. For photosynthetic analysis, mature leaves (with similar age) from eight different plants (randomly selected) were treated as biological replicates. When pools were used (e.g., carbohydrates and pigments analyses), each pool consisted of the two top mature leaves of eight different plants, and three replicates were used for each analysis. Three technical replicates were used for absorbance reading. All presented values are the mean ± standard deviation of the biological replicates from the independent experiments. Comparisons between all treatments and the control were made using the One-Way ANOVA test. When data were statistically different, the Dunnett Comparison Test ($p < 0.05$) was also applied. GraphpadTM Prism 7 was used. Multivariate analyses for data correlation used Principal Component Analysis and were performed with CANOCO V4.02 software for Windows.

3. Results

3.1. Shoot and Root Length, and Biomass

No significant differences were observed regarding the shoot length of inoculated seedlings. Treatment with 1PB1 and 5PB1 led to a significant decrease in root length compared to the control. However, 5PB1 treatment was not different from treatments with C9F and FV46. No strain treatment led to significant differences in root and shoot fresh and dry weight of seedlings compared to the control. However, C9F treatment led to a slight increase in fresh and dry weight of seedlings' roots (Table 1).

Table 1. Shoot and root length (cm), and weight (g) of 12 days-old seedlings under control conditions and inoculated with 1PB1, 5PB1, C9F, FV46 isolates. Values are means ± SD (n = 20) of measurements on each treatment. Means with the same letter are not significantly different according to the Dunnett test ($p < 0.05$). FW—fresh weight; DW—dry weight.

	Control	1PB1	5PB1	C9F	FV46
Shoot length	3.237 ± 0.491 a	3.147 ± 0.527 a	3.082 ± 0.513 a	3.100 ± 0.489 a	3.124 ± 0.491 a
Shoot FW	0.0609 ± 0.0139 a	0.0558 ± 0.0007 a	0.0561 ± 0.0097 a	0.0572 ± 0.0054 a	0.0568 ± 0.0118 a
Shoot DW	0.00256 ± 0.00053 a	0.00252 ± 0.00011 a	0.00251 ± 0.00045 a	0.00253 ± 0.00047 a	0.00257 ± 0.00056 a
Root length	9.163 ± 1.761 a	6.211 ± 1.195 c	7.382 ± 1.261 bc	8.271 ± 2.074 ab	8.082 ± 1.694 ab
Root FW	0.012 ± 0.005 a	0.012 ± 0.006 a	0.010 ± 0.006 a	0.013 ± 0.003 a	0.010 ± 0.004 a
Root DW	0.00053 ± 0.00005 a	0.00054 ± 0.00011 a	0.00049 ± 0.00012 a	0.00063 ± 0.00018 a	0.00056 ± 0.00002 a

3.2. Pigments Content

Regarding the effects of the strains on adult plants, no significant differences between treatments were observed in the contents of anthocyanins (Figure 1a) and carotenoids (Figure 1b) of inoculated plants, compared to the control group. Compared with the control, C9F led to a decrease and FV46 to an increase, in both Chl *a* (Figure 1c) and Chl *b* (Figure 1d). As these changes in Chl *a* and Chl *b* in plants inoculated by these isolates were proportional, the Chl *a/b* ratio was maintained (Figure 1e).

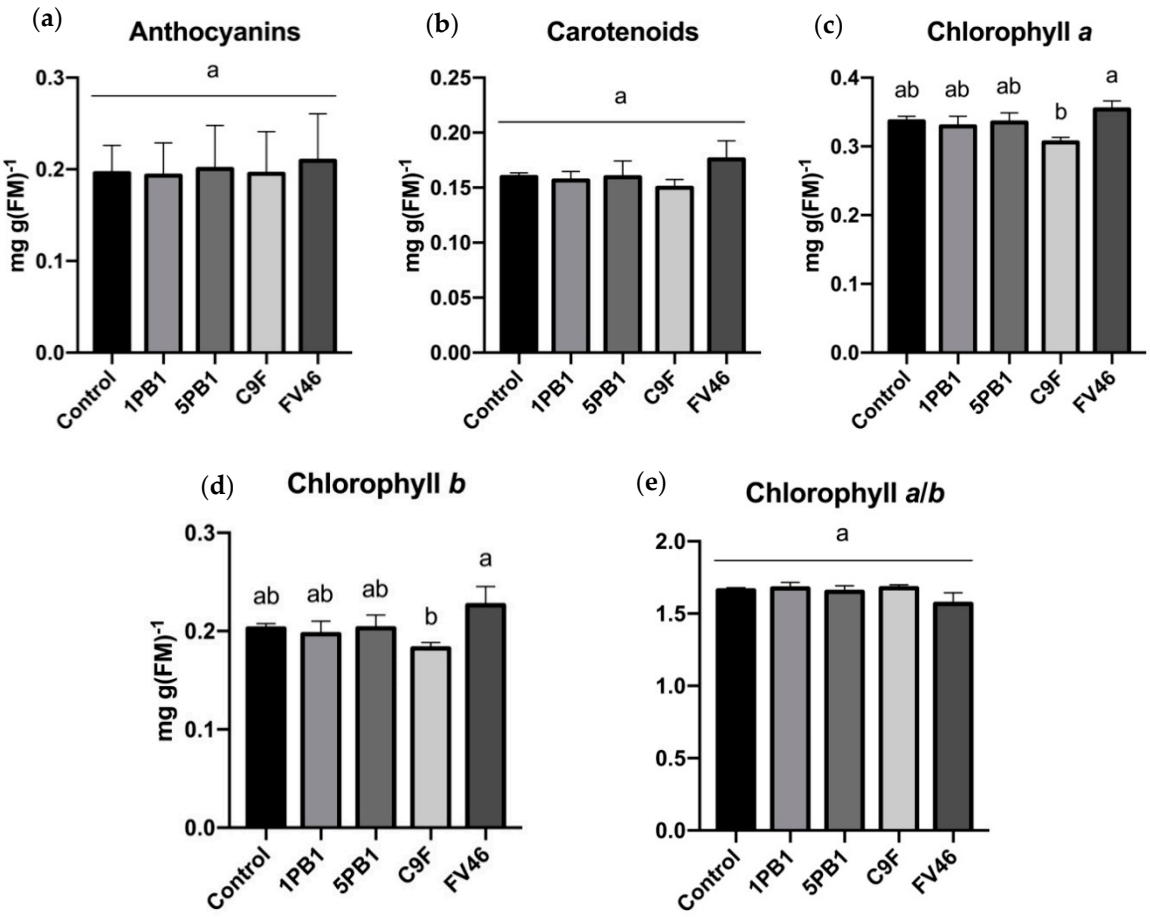

Figure 1. Effect of inoculation with isolates 1PB1, 5PB1, C9F and FV46 on tomato leaves of 130-day-old plants. (**a**) Anthocyanins, (**b**) Carotenoids; (**c**) Chl *a*, (**d**) Chl *b*, (**e**) Chl *a*/*b*. Presented values are means ± SD (n = 3) of measurements on each treatment group. Means with the same letter are not significantly different according to the Dunnett test ($p < 0.05$).

3.3. Carbohydrates Content

Plants inoculated with 1PB1 and C9F presented a higher TSS content compared to control (Figure 2a), and slightly higher TSS content compared to 5PB1 and FV46 treatments. The apparent increase in the TSS content of plants treated with 5PB1 and FV46 was not significantly different from the control group. Regarding starch (Figure 2b), plants treated with 1PB1 and 5PB1 presented a higher starch content than control plants. Additionally, 5PB1 inoculated plants had a higher starch content than plants inoculated with 1PB1.

3.4. Photosynthetic Performance

The maximum fluorescence (Fm) (Figure 3a) did not differ in plants inoculated with 5PB1 and FV46 compared to the control group. However, the inoculation of plants with C9F and 1PB1 significantly reduced Fm values. Considering the F_v/F_m ratio (Figure 3b), control plants showed a ratio of ~0.82, which was only lower in plants inoculated by C9F and FV46. Considering the F'_v/F'_m ratio of leaves adapted to light, results were similar to those in

dark-adapted leaves, with C9F significantly reducing this ratio compared to the control and 5PB1-treated plants. For qP and NPQ, the C9F-inoculated plants showed higher decreases compared to the controls. Regarding the efficiency of the PSII photosystem, inoculation with 1PB1, 5PB1 and FV46 resulted in no statistical differences, although 5PB1 lead to a slight increase. Inoculation with C9F significantly reduced the PSII efficiency.

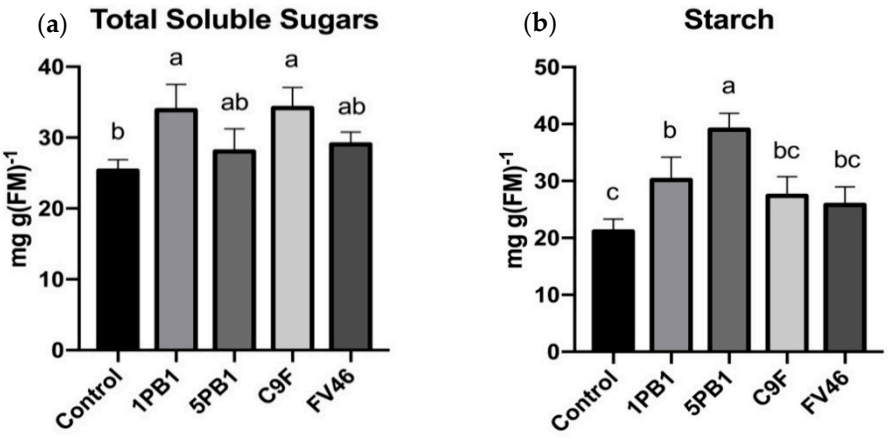

Figure 2. Effect of inoculation with isolates 1PB1, 5PB1, C9F and FV46 on Total Soluble Sugar (**a**) and Starch (**b**) contents, of tomato leaves of 130-day-old plants. Presented values are means ± SD (n = 3) of measurements on each treatment group. Means with the same letter are not significantly different according to the Dunnett test ($p < 0.05$).

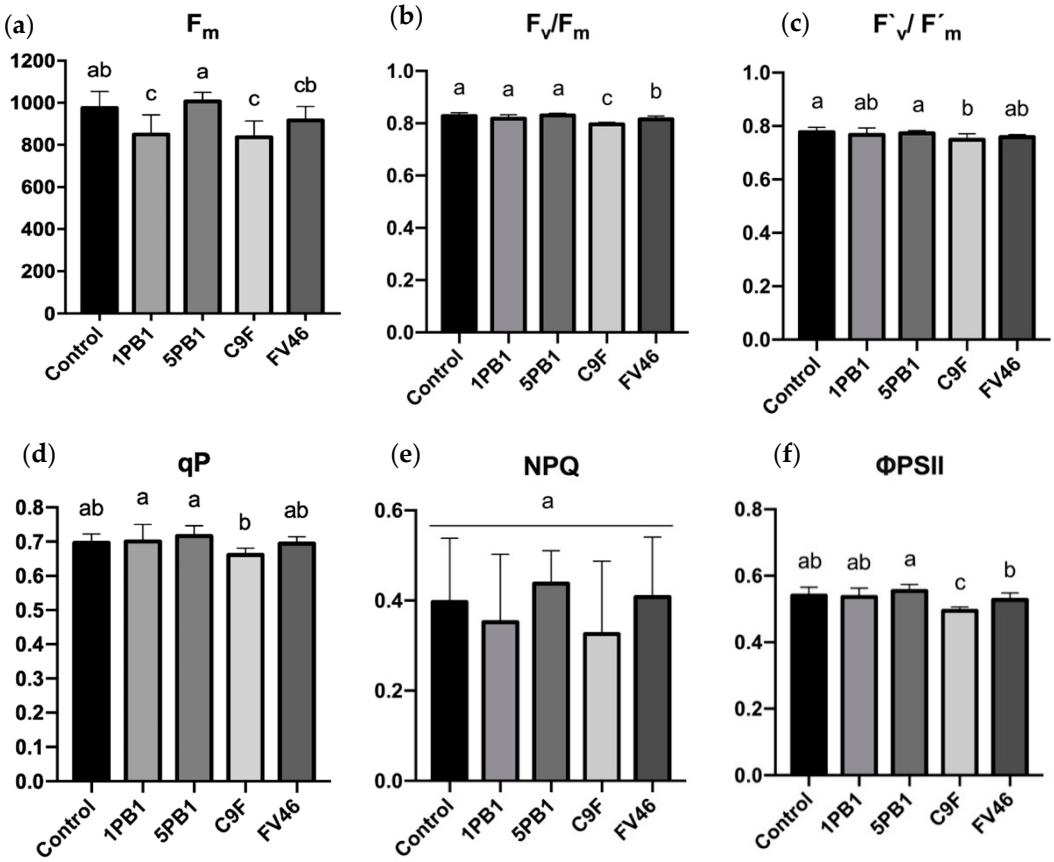

Figure 3. Effects of inoculation with isolates 1PB1, 5PB1, C9F and FV46 on PSII chlorophyll fluorescence of 130-day-old plants. Measurements on each treatment group were preformed (n = 8). Means with the same letter are not significantly different according to the Dunnett test ($p < 0.05$).

3.5. Gas Exchange

C9F decreased net CO_2 assimilation rate (P_N), while 1PB1, FV46 and 5PB1 did not significantly change P_N, although plants inoculated with 5PB1 had the highest P_N (Figure 4a). Stomatal conductance (gs) was significantly improved by 5PB1 compared to the control, while 1PB1, FV46, and C9F did not have significant effects (Figure 4c), which is in line with the change in the intercellular CO_2 concentration (Ci) (Figure 4b). Transpiration rate (E) was not affected by inoculations with 5PB1 or C9F, but it was significantly reduced by 1PB1 and FV46 (Figure 4d).

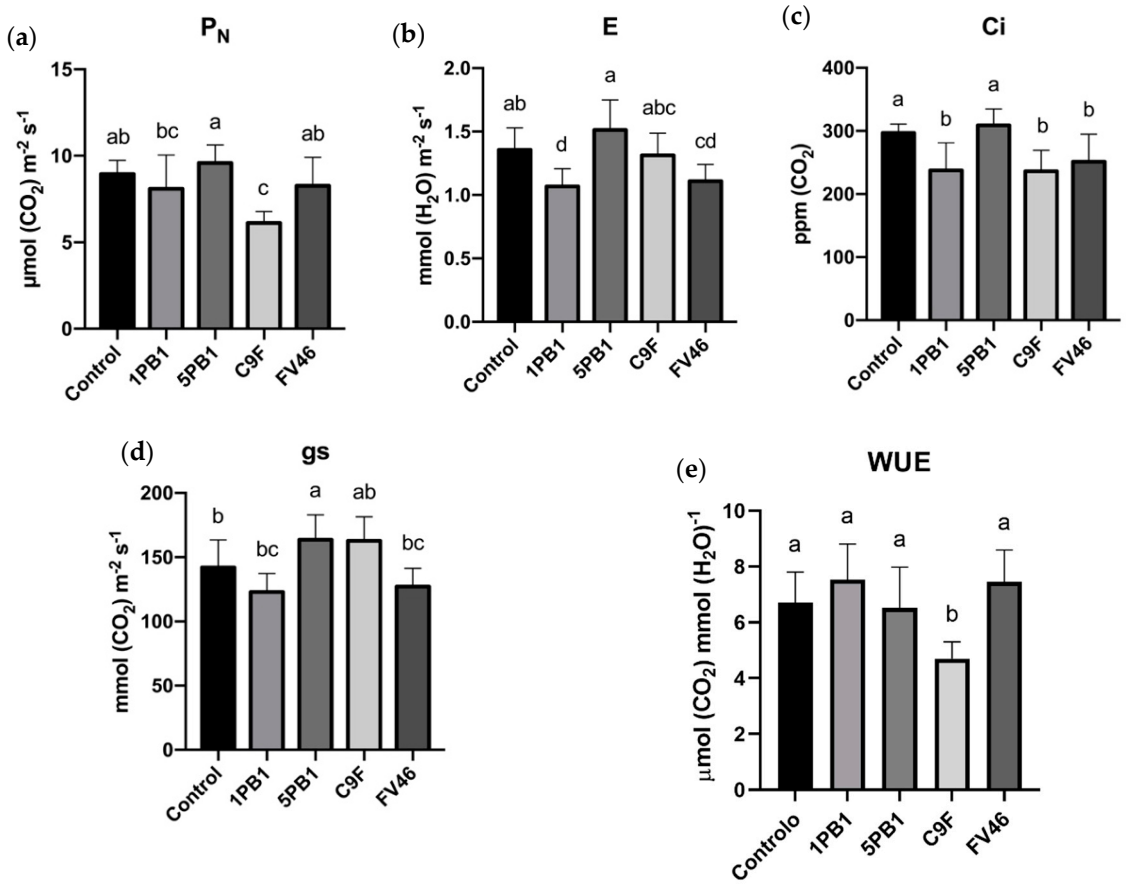

Figure 4. Effects of inoculation with 1PB1, 5PB1, C9F and FV46 on gas exchange parameters of 130-day-old tomato plants (n = 8); (**a**) PN—net photosynthetic rate; (**b**) Ci—internal CO_2 concentration; (**c**) gs—stomatal conductance; (**d**) E—transpiration rate; (**e**) WUE—water use efficiency. Means with the same letter are not significantly different according to the Dunnett test ($p < 0.05$).

3.6. Superoxide Content and Non-Enzymatic Antioxidant Activity

Inoculation of plants with 1PB1 and C9F resulted in lower superoxide concentration in leaves (Figure 5a). Inoculation of plants with FV46 resulted in significantly higher phenolic content compared to the control. Only slight increases were observed on plants inoculated with 1PB1 and C9F (Figure 5b). Results show that no significant differences regarding the DPPH free-radical-scavenging activity were observed among the conditions, although a slight increment was observed in plants inoculated with FV46 (Figure 5c).

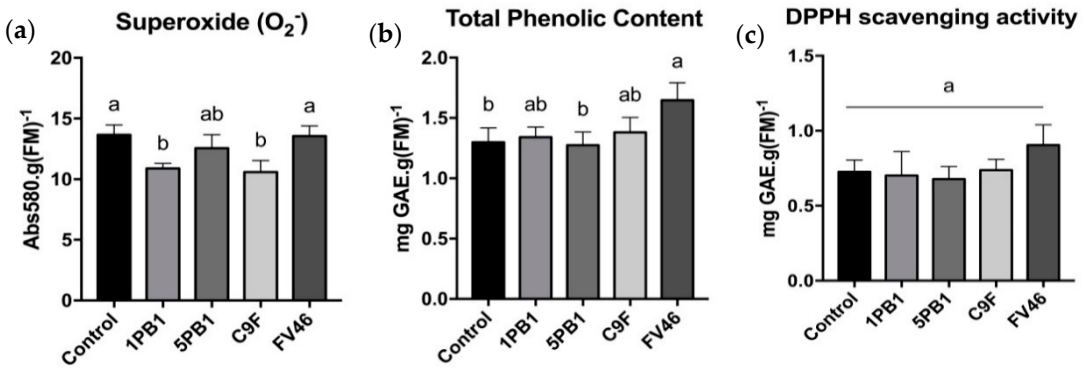

Figure 5. Effects of Inoculations with 1PB1, 5PB1, C9F and FV46 leaf redox status of 130 days-old tomato plants: (**a**) superoxide (O_2^-) content, (**b**) total phenolic content, (**c**) DPPH-scavenging activity. Presented values are means ± SD (n = 3) of measurements on each treatment group. Means with the same letter are not significantly different according to the Dunnett test ($p < 0.05$).

3.7. Principal Component Analysis (PCA)

The PCA showed a separation between control and inoculations, and a separation between the different strains (Figure 6).

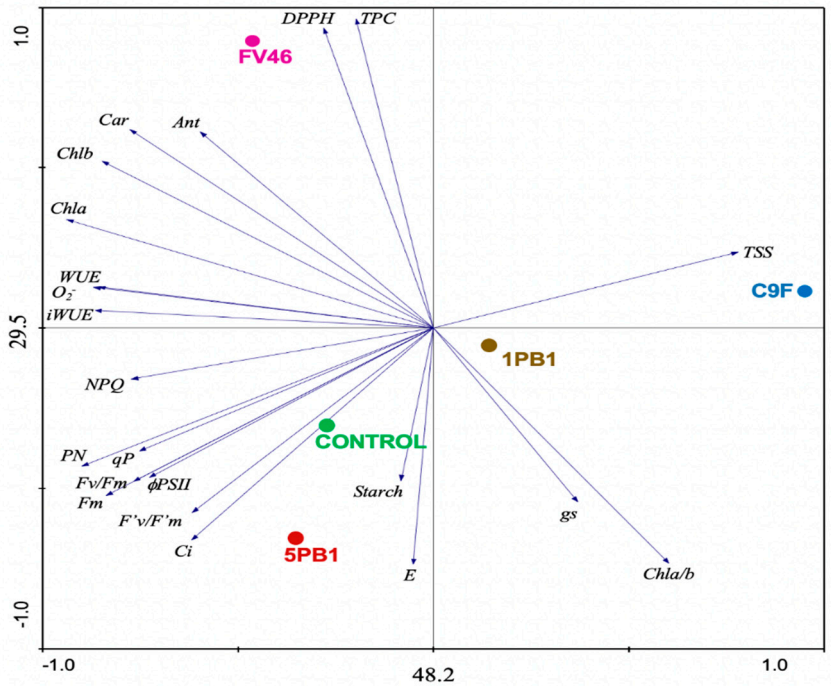

Figure 6. Principal component analysis biplot of the effects of inoculation of the isolates 1PB1, 5PB1, C9F and FV46 on tomato plants. Loading plot for the first axis PC1 explained 48.2% of the variance and second axis PC2 explained 29.5%. Ant—Anthocyanins; Car—Carotenoids; Chl *a*—Chlorophyll *a*; Chl *b*—Chlorophyll *b*; WUE—Water usage efficiency; O_2^-—Superoxide; NPQ—Nonphotochemical quenching; P_N—Net carbon assimilation rate; qP—Photochemical quenching; F_v/F_m—Maximum quantum efficiency of PSII; F_m—Maximum fluorescence; Φ_{PSII}—Quantum yield/Efficiency of PSII; F'_v/F'_m—Efficiency of excitation energy capture by open PSII reaction centers; Ci—internal CO_2; Starch—Starch content; E—Transpiration rate; gs—stomatal conductance; TSS—Total soluble sugars; TPC—Total phenolic content; DPPH—DPPH scavenging activity.

This separation is more accentuated between treatments with FV46, C9F and 1PB1 and the control. 5PB1 was the treatment that scored the closest to the control. PC1 explained 48.2% of the variance and PC2 explained 29.5%. FV46 treatment is located on the left upper

quadrant, correlating with pigments and non-enzymatic defense activity. 5PB1 is located on the lower left quadrant, along with the control, which was the group that mostly scored for photosynthetic and gas-exchange parameters, such as F_v/F_m, Φ_{PSII}, Ci and P_N. C9F is located on the upper right quadrant, scoring the highest for TSS. 1PB1 is located on the lower right quadrant, scoring for gs and Chl *a*/*b*.

4. Discussion

Plant-growth-promoting bacteria are known to improve plant growth and nutrition through the production of hormones, such as IAA, and by solubilizing soil nutrients [38]. These mechanisms end up influencing the physiological status of the plants. The present data showed that inoculation with different strains (selected as PGPB-candidates) changed the plants' photosynthesis and antioxidant responses.

The shoot length and fresh and dry weights of seedlings were not sensitive to the strains. 1PB1 and 5PB1 reduced root length, but the seedling's root maintained its FM and DM, meaning that the root increased its thickness. This thickening has been associated with some stress (e.g., mechanic), but we suggest that these strains (particularly 1PB1) may interfere with the root system architecture, by directly or indirectly influencing the levels of phytohormones (e.g., imbalances of indoleacetic-acid, gibberellic acid) [39]. Nonetheless, our results are in line with Cabra et al. [40], who demonstrated that *B. subtilis* GIBI 200 had little effect on the shoot and root length and biomass of treated tomato plants. Similarly, Gowtham et al. [41] showed that inoculation of tomato plants with several PGPB strains (including *Bacillus* spp.) did not lead to changes in shoot and root length in plants grown under controlled conditions, but induced some increases in these parameters in plants growing under greenhouse conditions.

Regarding the adult plants, the strains showed bioactivity in terms of both photosynthesis and redox status. The F_v/F_m that estimates the maximum quantum efficiency of PSII photochemistry varied between 0.79 and 0.83, which is in agreement with values described in the literature for healthy unstressed plants [42], supporting no negative effects of these strains on the photosynthetic performance. These results are in line with the work of Yobo et al. [43], who found that the inoculation of dry bean seedlings with *Bacillus* spp. isolates leads to a slight increase in maximum PSII efficiency (F_v/F_m), although that difference was not maintained with time, compared to control plants. Similarly, Anusaraporn et al. [44] demonstrated that inoculation of rice plants with *Bacillus* sp. N7 did not change this ratio. Regarding the F'_v/F'_m ratio, which estimates the maximum PSII efficiency when all the PSII centres are "open" (in light-adapted leaves), only the C9F treatment lowered this ratio. As far as we know, there are no data in the literature regarding PGPB-mediated changes in the PSII-effective efficiency (F'_v/F'_m) of tomato plants. However, in other species (e.g., *Euterpe oleracea*), De Castro et al. [45] demonstrated that *B. subtilis* combined with *B. safensis* and other PGPB lead to a significant increase in this parameter. Photochemical quenching (qP) was not affected by the inoculations, although a tendency towards reduction was observed in plants inoculated with C9F, and a tendency to increase the proportion of PSII reaction centres that are open was observed in plants treated with 5PB1 and 1PB1. Furthermore, 5PB1 and FV46 isolates may be interesting for use to promote plants' Φ_{PSII}, which translates the effective quantum yield of the PSII electron transport. The PCA supports these findings, particularly for the 5PB1 treatment which is positioned in the same quadrant as the control, near Φ_{PSII} (Figure 6). Similar results were reported by Li et al. [46], who found an increase in Φ_{PSII} on *V. faba* plants inoculated with *B. subtilis*, but, in contrast, Jain and Jajoo [47] verified a slight decrease in Φ_{PSII} on wheat plants treated with *B. subtilis* (NCIM 5594). In our work, only the treatment with C9F negatively influenced the Φ_{PSII}, which was also confirmed by the PCA. This reduction in Φ_{PSII} was related to the decrease in open PSII reaction centers (qP) and/or to the reduction in the efficiency of excitation capture by open PSII reaction centers (F'_v/F'_m), as supported by the PCA (positive correlation between these parameters). Moreover, a decrease in Φ_{PSII} can result in a low availability of ATP and NADPH for the Calvin Cycle. Therefore, the lack of

beneficial effects in plants inoculated with 1PB1 and C9F might be explained by the fact that these isolates were not obtained from the rhizosphere of tomato plants, so host-specificities might explain this fact, as was already demonstrated by Vaikuntapu et al. [48]. On the other hand, 5PB1 and FV46 seem to have some beneficial effects on a broader range of plant species. Non-photochemical quenching (NPQ), which monitors the apparent rate for heat loss from PSII, was unaffected, suggesting that these isolates do not affect this protective mechanism. Different results were reported by Samaniego-Gámez et al. [49], who found the inoculation of pepper plants with *Bacillus* sp. M9 and *B. cereus* K46 decreased NPQ, but with an increase of qP. Similarly, Li et al. [46] found that *B. subtillis* increased NPQ in *V. faba* plants.

In line with the Φ_{PSII} data, gas exchange parameters also might be positively influenced by 5PB1, and the PCA confirms this finding (Figure 6). The effect of 5PB1 on the P_N in tomato plants may be due to the lower restriction in E and gs, and consequent high CO_2 availability (Ci). On the other hand, the restrictions observed on the Φ_{PSII} of C9F-treated plants may also explain the decline of $P_{N,}$ as supported by the multivariate analysis (Figure 6). As a consequence, WUE was decreased. Contrarily, the other inoculants did not influence WUE. Different results were reported by Samaniego-Gámez et al. [49], who demonstrated that the inoculation of pepper plants with two *Bacillus* spp. strains significantly increased WUE.

In the PCA analysis, FV46 score positioned in the upper left quadrant, close to chlorophylls (Figure 6), thus supporting a positive correlation. Our data are in agreement with the reported by Akram et al. [50], who showed that a *B. megaterium* strain increased the levels of Chl *a*, Chl *b,* and carotenoids in tomato plants. Shah et al. [51] obtained similar results when inoculating tomato plants with four different *Bacillus* isolates. This positive effect is common for a wide range of species. The 5PB1 and 1PB1 treatments had little influence on pigments, while C9F slightly decreased pigment levels. These results are in line with Morais et al. [52] who showed that the inoculation of strawberry plants with isolates of *Pedobacter* sp. CC1, *B. safensis* B106, and *B. subtilis* B167A exerted low or no impact on pigment content.

All isolates in general increased the leaves' carbohydrates, with 1PB1 and 5PB1 promoting a particularly significant accumulation of starch, and 1PB1 and C9F an increase in TSS pool. PCA also confirms that 5PB1 had a stronger positive influence on starch content, and 1PB1 and C9F on TSS. These changes may demonstrate a strain-dependent influence on the plant's carbon metabolism and/or photo-assimilates' translocation, which deserves further study. Gagné-Bourque et al. (2015) showed that inoculation of *Brachypodium distachyon* with *B. subtilis* B26 stimulated the accumulation of starch and TSS just after 8 days of growth [53]. In the present work, the accumulation of TSS verified in plants inoculated with C9F might be linked to the observed decrease in P_N. This finding is also supported by the negative correlation between these parameters observed in the PCA, possibly indicating a reduction in the use of TSS. We have found that isolates 1PB1 and C9F produce more IAA than 5PB1 and FV46 (data not shown), and bacterial IAA could justify the increase in carbohydrates in these plants [54]. It is also known that plants usually provide sugars to beneficial bacteria, via root exudates, so higher sugar production may be linked to such an interaction [38]. Moreover, the accumulation of soluble sugars helps plants keep homeostasis, namely under drought [53]. The putative effects of these isolates to help plants tolerate drought thus deserves further study.

The treatments with 1PB1 and C9F reduced the levels of superoxide content, supporting an additional perspective for the benefits of these isolates in the redox protection of plants. Reducing the levels of superoxide is advantageous for plants facing adverse environmental conditions. Moreover, it is known that bacteria produce defense enzymes such as superoxide dismutase (SOD) [55], which could explain the lower superoxide contents in plants treated with these isolates, and support their potential benefits. This negative correlation between superoxide and 1PB1- and C9F-treated plants was also confirmed by the PCA. Regarding total phenolic content (TPC), the inoculant FV46 favored the accu-

mulation of these compounds. These results are in line with Abd-Allah et al. [56], who showed that a *B. subtilis* isolate led to an increase in the TPC in chickpea plants. Since phenolic contents have reducing properties and play a role in scavenging free radicals [57], the results suggest that FV46 might improve non-enzymatic antiradical defenses. The improvement in chlorophyll content by FV46 might be related to some protection provided by the TPC, which is also supported by the PCA.

5. Conclusions

Finding new PGPB species/strains that can be used as sustainable alternatives to current agrochemicals is gaining relevance. Here, we tested the effects of new isolates on the model cv. MicroTom growth of seedlings and adult plants' physiology. These strains mostly affected adult plants, but the effects depend on the strain. *B. laterosporus* C9F negatively influenced the levels of chlorophylls, PSII chlorophyll fluorescence, and some parameters of gas exchange. The 5PB1 showed the most positive correlation with PSII chlorophyll fluorescence, and *B. safensis* FV46 was the most efficient in stimulating some plant antioxidant defenses (e.g., phenols), suggesting a protective action against oxidative stress. We are currently using combinations of these isolates to test if they can act synergistically and provide plants with different benefits. Assessment the effects of these strains on different commercial cultivars is being initialized. These isolates can modulate the physiology of species that are different from their natural hosts, which may be a desirable characteristic in terms of field/agricultural application and commercialization. However, additional studies with different plant species should be conducted to draw further conclusions.

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References

1. Brown, K.H.; Jameton, A.L. Public health implications of urban agriculture. *J. Public Health Policy* **2000**, *21*, 20–39. [CrossRef] [PubMed]
2. Brühl, C.A.; Zaller, J.G. Biodiversity decline as a consequence of an inadequate environmental risk assessment of pesticides. *Front. Environ. Sci.* **2019**, *7*, 177. [CrossRef]
3. Singh, J.; Yadav, A.N. (Eds.) *Natural Bioactive Products in Sustainable Agriculture*; Springer: Singapore, 2020. [CrossRef]
4. Basu, S.; Rabara, R.; Negi, S. Towards a better greener future-an alternative strategy using biofertilizers. I: Plant growth promoting bacteria. *Plant Gene* **2017**, *12*, 43–49. [CrossRef]
5. Chandra, D.; Barh, A.; Sharma, I.P. Plant growth promoting bacteria: A gateway to sustainable agriculture. In *Microbial Biotechnology in Environmental Monitoring and Cleanup*; IGI Global: Hershey, PA, USA, 2018; pp. 318–338. [CrossRef]
6. Khan, A.L.; Waqas, M.; Kang, S.-M.; Al-Harrasi, A.; Hussain, J.; Al-Rawahi, A.; Al-Khiziri, S.; Ullah, I.; Ali, L.; Jung, H.-Y.; et al. Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. *J. Microbiol.* **2014**, *52*, 689–695. [CrossRef]
7. Etesami, H.; Alikhani, H.A.; Hosseini, H.M. Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. *MethodsX* **2015**, *2*, 72–78. [CrossRef] [PubMed]
8. Sharma, A.; Johri, B.N. Growth promoting influence of siderophore-producing Pseudomonas strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiol. Res.* **2003**, *158*, 243–248. [CrossRef] [PubMed]

9. Gull, M.; Hafeez, F.Y. Characterization of siderophore producing bacterial strain Pseudomonas fluorescens Mst 8.2 as plant growth promoting and biocontrol agent in wheat. *Afr. J. Microbiol. Res.* **2012**, *6*, 6308–6318. [CrossRef]
10. Yadegari, M.; Rahmani, H.A.; Noormohammadi, G.; Ayneband, A. Plant growth promoting rhizobacteria increase growth, yield and nitrogen fixation in *Phaseolus vulgaris*. *J. Plant Nutr.* **2010**, *33*, 1733–1743. [CrossRef]
11. Gopalakrishnan, S.; Srinivas, V.; Samineni, S. Nitrogen fixation, plant growth and yield enhancements by diazotrophic growth-promoting bacteria in two cultivars of chickpea (*Cicer arietinum* L.). *Biocatal. Agric. Biotechnol.* **2017**, *11*, 116–123. [CrossRef]
12. Kang, S.-M.; Joo, G.-J.; Hamayun, M.; Na, C.-I.; Shin, D.-H.; Kim, H.Y.; Hong, J.-K.; Lee, I.-J. Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnol. Lett.* **2008**, *31*, 277–281. [CrossRef]
13. Midekssa, M.J.; Löscher, C.R.; Schmitz, R.A.; Assefa, F. Phosphate solubilization and multiple plant growth promoting properties of rhizobacteria isolated from chickpea (*Cicer aeritinum* L.) producing areas of Ethiopia. *Afr. J. Biotechnol.* **2016**, *15*, 1899–1912. [CrossRef]
14. Mnif, I.; Ghribi, D. Potential of bacterial derived biopesticides in pest management. *Crop Prot.* **2015**, *77*, 52–64. [CrossRef]
15. Mnif, I.; Hammami, I.; Triki, M.A.; Azabou, M.C.; Ellouze-Chaabouni, S.; Ghribi, D. Antifungal efficiency of a lipopeptide biosurfactant derived from *Bacillus subtilis* SPB1 versus the phytopathogenic fungus, *Fusarium solani*. *Environ. Sci. Pollut. Res.* **2015**, *22*, 18137–18147. [CrossRef] [PubMed]
16. Miethke, M.; Marahiel, M.A. Siderophore-Based iron acquisition and pathogen control. *Microbiol. Mol. Biol. Rev.* **2015**, *71*, 413–451. [CrossRef] [PubMed]
17. Jain, S.; Varma, A.; Tuteja, N.; Choudhary, D.K. Plant growth-promoting microbial-mediated induced systemic resistance in plants: Induction, mechanism, and expression. In *Microbial-Mediated Induced Systemic Resistance in Plants*; Springer: Singapore, 2016; pp. 213–226. [CrossRef]
18. Jiménez-Gómez, A.; García-Estévez, I.; García-Fraile, P.; Escribano-Bailón, M.T.; Rivas, R. Increase in phenolic compounds of *Coriandrum sativum* L. after the application of a *Bacillus halotolerans* biofertilizer. *J. Sci. Food Agric.* **2020**, *100*, 2742–2749. [CrossRef] [PubMed]
19. Danish, S.; Zafar-ul-Hye, M. Co-Application of ACC-deaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress. *Sci. Rep.* **2019**, *9*, 1–13. [CrossRef]
20. Xie, L.; Lehvävirta, S.; Timonen, S.; Kasurinen, J.; Niemikapee, J.; Valkonen, J.P.T. Species-specific synergistic effects of two plant growth—promoting microbes on green roof plant biomass and photosynthetic efficiency. *PLoS ONE* **2018**, *13*, e0209432. [CrossRef] [PubMed]
21. Shi, Y.; Lou, K.; Li, C. Growth and photosynthetic efficiency promotion of sugar beet (*Beta vulgaris* L.) by endophytic bacteria. *Photosynth. Res.* **2010**, *105*, 5–13. [CrossRef]
22. Stefan, M.; Munteanu, N.; Stoleru, V.; Mihasan, M.; Hritcu, L. Seed inoculation with plant growth promoting rhizobacteria enhances photosynthesis and yield of runner bean (*Phaseolus coccineus* L.). *Sci. Hortic.* **2013**, *151*, 22–29. [CrossRef]
23. Wang, C.-J.; Yang, W.; Wang, C.; Gu, C.; Niu, D.-D.; Liu, H.-X.; Wang, Y.; Guo, J.-H. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting Rhizobacterium strains. *PLoS ONE* **2012**, *7*, e52565. [CrossRef]
24. Vimal, S.R.; Patel, V.K.; Singh, J.S. Plant growth promoting *Curtobacterium albidum* strain SRV4: An agriculturally important microbe to alleviate salinity stress in paddy plants. *Ecol. Indic.* **2018**, *105*, 553–562. [CrossRef]
25. Khanna, K.; Jamwal, V.L.; Gandhi, S.G.; Ohri, P.; Bhardwaj, R. Metal resistant PGPR lowered Cd uptake and expression of metal transporter genes with improved growth and photosynthetic pigments in *Lycopersicon esculentum* under metal toxicity. *Sci. Rep.* **2019**, *9*, 1–14. [CrossRef] [PubMed]
26. Han, H.S.; Lee, K.D. 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.
27. Instituto Português da Atmosfera e do Mar. Boletim Climatológico Mensal—Julho de 2020. 2020. Available online: http://www.ipma.pt/pt/media/noticias/documentos/2020/resumo_clima_mar2020.pdf (accessed on 10 October 2020).
28. Instituto Português da Atmosfera e do Mar. Resumo Climatológico Março de 2020. 2020. Available online: https://www.ipma.pt/pt/media/noticias/news.detail.jsp?f=/pt/media/noticias/textos/resumo-clima-julho-20.html (accessed on 10 October 2020).
29. Sims, D.A.; Gamon, J.A. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.* **2002**, *81*, 337–354. [CrossRef]
30. Irigoyen, J.J.; Einerich, D.W.; Sánchez-Díaz, M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant.* **1992**, *84*, 55–60. [CrossRef]
31. Osaki, M.; Shinano, T.; Tadano, T. Redistribution of carbon and nitrogen compounds from the shoot to the harvesting organs during maturation in field crops. *Soil Sci. Plant Nutr.* **1991**, *37*, 117–128. [CrossRef]
32. Dias, M.C.; Santos, C.; Pinto, G.; Silva, A.M.; Silva, S. Titanium dioxide nanoparticles impaired both photochemical and non-photochemical phases of photosynthesis in wheat. *Protoplasma* **2019**, *256*, 69–78. [CrossRef]
33. Klughammer, C.; Schreiber, U. Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. *PAM Appl. Notes* **2008**, *1*, 201–247.
34. Murchie, E.H.; Lawson, T. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *J. Exp. Bot.* **2013**, *64*, 3983–3998. [CrossRef]

35. Dewanto, V.; Wu, X.; Adom, K.K.; Liu, R.H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014. [CrossRef]
36. Harkat-Madouri, L.; Asma, B.; Madani, K.; Said, Z.B.-O.S.; Rigou, P.; Grenier, D.; Allaloua, H.; Remini, H.; Adjaouda, A.; Boulekbache-Makhlouf, L. Chemical composition, antibacterial and antioxidant activities of essential oil of *Eucalyptus globulus* from Algeria. *Ind. Crop. Prod.* **2015**, *78*, 148–153. [CrossRef]
37. Gajewska, E.; Sklodowska, M. Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. *BioMetals* **2007**, *20*, 27–36. [CrossRef] [PubMed]
38. Olanrewaju, O.S.; Ayangbenro, A.S.; Glick, B.R.; Babalola, O.O. Plant health: Feedback effect of root exudates-rhizobiome interactions. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 1155–1166. [CrossRef] [PubMed]
39. Jung, J.K.; McCouch, S. Getting to the roots of it: Genetic and hormonal control of root architecture. *Front. Plant Sci.* **2013**, *4*, 186. [CrossRef]
40. Cabra Cendales, T.; Rodríguez González, C.A.; Villota Cuásquer, C.P.; Tapasco Alzate, O.A.; Hernández Rodríguez, A. Bacillus effect on the germination and growth of tomato seedlings (*Solanum lycopersicum* L.). *Acta Biol. Colomb.* **2017**, *22*, 37. [CrossRef]
41. Gowtham, H.G.; Duraivadivel, P.; Hariprasad, P.; Niranjana, S.R. A novel split-pot bioassay to screen indole acetic acid producing rhizobacteria for the improvement of plant growth in tomato (*Solanum lycopersicum* L.). *Sci. Hortic.* **2017**, *224*, 351–357. [CrossRef]
42. Zhori, A.; Meco, M.; Brandl, H.; Bachofen, R. In situ chlorophyll fluorescence kinetics as a tool to quantify effects on photosynthesis in Euphorbia cyparissias by a parasitic infection of the rust fungus *Uromyces pisi*. *BMC Res. Notes* **2015**, *8*, 698. [CrossRef]
43. Yobo, K.S.; Laing, M.D.; Hunter, C.H. Effects of single and combined inoculations of selected Trichoderma and *Bacillus* isolates on growth of dry bean and biological control of *Rhizoctonia solani* damping-off. *Afr. J. Biotechnol.* **2011**, *10*, 8746–8756.
44. Anusaraporn, S.; Autarmat, S.; Treesubsuntorn, C.; Thiravetyan, P. Application *Bacillus* sp. N7 to enhance ozone tolerance of various *Oryza sativa* in vegetative phase: Possible mechanism and rice productivity. *Biocatal. Agric. Biotechnol.* **2020**, *25*, 101591. [CrossRef]
45. de Castro, G.L.S.; Rêgo, M.C.F.; Silvestre, W.V.D.; Batista, T.F.V.; da Silva, G.B. Açaí palm seedling growth promotion by rhizobacteria inoculation. *Braz. J. Microbiol.* **2020**, *51*, 205–216. [CrossRef]
46. Li, Y.; Xu, S.; Gao, J.; Pan, S.; Wang, G. *Bacillus subtilis*-regulation of stomatal movement and instantaneous water use efficiency in *Vicia faba*. *Plant Growth Regul.* **2015**, *78*, 43–55. [CrossRef]
47. Jain, L.; Jajoo, A. Protection of PSI and PSII complexes of wheat from toxic effect of anthracene by *Bacillus subtilis* (NCIM 5594). *Photosynth. Res.* **2020**, *146*, 197–211. [CrossRef] [PubMed]
48. Vaikuntapu, P.R.; Dutta, S.; Samudrala, R.B.; Rao, V.R.V.N.; Kalam, S.; Podile, A.R. Preferential promotion of *Lycopersicon esculentum* (Tomato) Growth by plant growth promoting bacteria associated with tomato. *Indian J. Microbiol.* **2014**, *54*, 403–412. [CrossRef] [PubMed]
49. Samaniego-Gámez, B.Y.; Garruña, R.; Tun-Suárez, J.M.; Kantun-Can, J.; Reyes-Ramírez, A.; Cervantes-Díaz, L. *Bacillus* spp. inoculation improves photosystem II efficiency and enhances photosynthesis in pepper plants. *Chil. J. Agric. Res.* **2016**, *76*, 409–416. [CrossRef]
50. Akram, W.; Aslam, H.; Ahmad, S.R.; Anjum, T.; Yasin, N.A.; Khan, W.U.; Ahmad, A.; Guo, J.; Wu, T.; Luo, W.; et al. *Bacillus megaterium* strain A12 ameliorates salinity stress in tomato plants through multiple mechanisms. *J. Plant Interact.* **2019**, *14*, 506–518. [CrossRef]
51. Shah, R.; Amaresan, N.; Patel, P.; Jinal, H.N.; Krishnamurthy, R. Isolation and characterization of *Bacillus* spp. endowed with multifarious plant growth-promoting traits and their potential effect on tomato (*Lycopersicon esculentum*) seedlings. *Arab. J. Sci. Eng.* **2020**, *45*, 4579–4587. [CrossRef]
52. Morais, M.C.; Mucha, Â.; Ferreira, H.; Gonçalves, B.; Bacelar, E.; Marques, G. Comparative study of plant growth-promoting bacteria on the physiology, growth and fruit quality of strawberry. *J. Sci. Food Agric.* **2019**, *99*, 5341–5349. [CrossRef] [PubMed]
53. Gagné-Bourque, F.; Mayer, B.F.; Charron, J.-B.; Vali, H.; Bertrand, A.; Jabaji, S. Accelerated growth rate and increased drought stress resilience of the model grass *Brachypodium distachyon* Colonized by *Bacillus subtilis* B26. *PLoS ONE* **2015**, *10*, e0130456. [CrossRef]
54. Li, J.; Guan, Y.; Yuan, L.; Hou, J.; Wang, C.; Liu, F.; Yang, Y.; Lu, Z.; Chen, G.; Zhu, S. Effects of exogenous IAA in regulating photosynthetic capacity, carbohydrate metabolism and yield of *Zizania latifolia*. *Sci. Hortic.* **2019**, *253*, 276–285. [CrossRef]
55. Liu, P.; Ewis, H.E.; Huang, Y.J.; Lu, C.D.; Tai, P.C.; Weber, I.T. Structure of *Bacillus subtilis* superoxide dismutase. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **2007**, *63*, 1003–1007. [CrossRef] [PubMed]
56. Abd_Allah, E.F.; Alqarawi, A.A.; Hashem, A.; Radhakrishnan, R.; Al-Huqail, A.A.; Al-Otibi, F.O.N.; Malik, J.A.; Alharbi, I.; Egamberdieva, D. Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J. Plant Interact.* **2018**, *13*, 37–44. [CrossRef]
57. Prakash, D.; Singh, B.N.; Upadhyay, G. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chem.* **2007**, *102*, 1389–1393. [CrossRef]