Effects of Rhizosphere Bacteria on Strawberry Plants
(Fragaria × ananassa Duch.) under Water Deficit

Dominika Paliwoda 1, Grzegorz Mikiciuk 1,*, Małgorzata Mikiciuk 2, Anna Kisiel 3,4, Lidia Sas-Paszt 5
and Tymoteusz Miller 3,4

1 Department of Horticulture, Faculty of Environmental Management and Agriculture,
West Pomeranian University of Technology in Szczecin, Słowackiego 17, 71-434 Szczecin, Poland
2 Department of Bioengineering, Faculty of Environmental Management and Agriculture,
West Pomeranian University of Technology in Szczecin, Słowackiego 17, 71-434 Szczecin, Poland
3 Institute of Marine and Environmental Sciences, University of Szczecin, Wąska 13, 71-415 Szczecin, Poland
4 Polish Society of Bioinformatics and Data Science BIODATA, Popiełuszki 4c, 71-214 Szczecin, Poland
5 Department of Microbiology and Rhizosphere, The National Institute of Horticultural Research,
Pomologiczna 18, 96-100 Skiermiewice, Poland

* Correspondence: grzegorz.mikiciuk@zut.edu.pl

Abstract: Due to the observed climate warming, water deficiency in soil is currently one of the most
important stressors limiting the size and quality of plant crops. Drought stress causes a number of
morphological, physiological, and biochemical changes in plants, limiting their growth, development,
and yield. Innovative methods of inducing resistance and protecting plants against stressors include
the inoculation of crops with beneficial microorganisms isolated from the rhizosphere of the plant
species to which they are to be applied. The aim of the present study was to evaluate 12 different
strains of rhizosphere bacteria of the genera Pantoea, Bacillus, Azotobacter, and Pseudomonas by using
them to inoculate strawberry plants and assessing their impact on mitigating the negative effects of
drought stress. Bacterial populations were assessed by estimates of their size based on bacterial
counts in the growth substrate and with bioassays for plant growth-promoting traits. The physiological
condition of strawberry plants was determined based on the parameters of chlorophyll fluorescence.
The usefulness of the test methods used to assess the influence of plant inoculation with rhizosphere
bacteria on the response of plants growing under water deficit was also evaluated. A two-factor
experiment was performed in a complete randomization design. The first experimental factor was the
inoculation of plant roots with rhizosphere bacteria. The second experimental factor was the
different moisture content of the growth substrate. The water potential was maintained at −10 to
−15 kPa under control conditions, and at −40 to −45 kPa under the conditions of water deficit in
the substrate. The tests on strawberry plants showed that the highest sensitivity to water deficiency,
and thus the greatest usefulness for characterizing water stress, was demonstrated by the following
indices of chlorophyll “a” fluorescence: F_M, F_V, F_V/F_M, PI, and Area. Based on the assessment of the
condition of the photosynthetic apparatus and the analysis of chlorophyll “a” fluorescence indices,
including hierarchical cluster analysis, the following strains of rhizosphere bacteria were found to
have favorable effects on strawberry plants under water deficit: the Bacillus sp. strains DLGB2 and
DKB26 and the Pantoea sp. strains DKB63, DKB70, DKB68, DKB64, and DKB65. In the tests, these
strains of Bacillus sp. exhibited a common trait—the ability to produce siderophores, while those of
Pantoea sp. were notable for phosphate mobilization and ACCD activity.

Keywords: fluorescence of chlorophyll “a”; bacterial counts; drought stress; PGPR; strawberry

1. Introduction

Due to the observed climate warming, water deficiency in soil is currently one of the
most important stressors limiting the size and quality of plant crops. On a global scale,
drought affects one-third of soils [1]. In modern agriculture, the unfavorable impact of
commonly used pesticides and chemical fertilizers on limiting the microbiological diversity of soils also constitutes a major problem [2].

Soil water deficit may lead to, inter alia, a reduction in the efficiency of photosynthesis through its influence on the functioning of the stomata and on the process of accumulation and transport of assimilates [3,4]. Stress initiates the operation of mechanisms of scattering the excess absorbed light energy, one of them being the fluorescence of chlorophyll “a”. Hence, research methods and techniques based on this phenomenon are among the most sensitive of those used to identify stress states in plants. They are also non-invasive and rapid. They are considered to be useful methods for the assessment of plant tolerance to environmental stressors [5].

In order to manage water most rationally, both for economic and ecological reasons, new solutions are sought to reduce the impact of water deficit on crops. Innovative methods of inducing resistance and protecting plants against stressors include the inoculation of crops with beneficial microorganisms isolated from the rhizosphere of the plant species to which they are to be applied. Groups of rhizosphere microorganisms and their interactions are the subject of research aimed at determining their influence on the growth, yield, and protection of plants [6–8]. These organisms colonize the roots of plants and induce growth and immune processes in them, which can contribute to the neutralization or minimization of the impact of stresses caused by, for example, water deficiency [9–12], high temperature [13,14], low temperature [15], salinity [16,17], soil contamination with heavy metals [18,19], or biotic factors (pathogens) [20,21]. Among free-living bacteria are Plant Growth Promoting Rhizobacteria (PGPR), which have been utilized for improving water and nutrient uptake and abiotic and biotic stress tolerance [22,23]. Among the mechanisms involved, there are: phosphate mobilization, ammonia production, organic acids production, nitrogenase activity, biofilm formation, the production of siderophores, exopolysaccharides, phytohormones (including indole-3-acetic acid—I AA), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity [24–27]. Many studies have shown enhanced stress tolerance in plants through inoculation with PGPR. PGPRs secrete ACC deaminase, which destroys an ethylene precursor ACC, protecting plants against drought stress [28–31]. It has been reported that auxin-producing bacteria also protect plants from stress by increasing root length, the number of root tips, root surface area, and total plant biomass. As a result, they increase the uptake of water and nutrients and stimulate the synthesis of ACC deaminase [32,33]. Bacteria promoting plant growth and yielding under both normal and stressful conditions include, among others: Azospirillum, Arthrobacter, Bacillus, Enterobacter, Pseudomonas, Rhizobium, Pantoea, and Azoarcus [2,34–36].

The strawberry is an economically important berry species [37] that is characterized by high sensitivity to water deficiency [38–40]. This species is therefore a very good object of research on the effects of factors that can potentially relieve drought stress, including the effectiveness of inoculating plants with plant growth-promoting bacteria.

The aim of the present study was to evaluate 12 strains of rhizosphere bacteria of the genera Pantoea, Bacillus, Azotobacter, and Pseudomonas by using them to inoculate strawberry plants and assessing their impact on mitigating the negative effects of drought stress. Bacterial populations were assessed by estimating their size based on bacterial counts in the growth substrate and with bioassays for plant growth-promoting traits. The physiological condition of strawberry plants was determined based on the parameters of chlorophyll fluorescence. The research results may be used for selecting PGPR strains with the highest anti-stress potential to reduce the potential adverse effects of water deficit on the growth and yielding of plants.

The usefulness of the test methods used to assess the influence of plant inoculation with rhizospheric bacteria on the response of plants growing under water deficit was also evaluated.
2. Results

2.1. Bioassays for Plant Growth Promoting Traits

All of the strains reported on in the present study exhibited PGP traits in the biochemical assays. Among the tested strains, only two (Azotobacter sp. AJ 1.1 and Pseudomonas sp. PJ 1.2) manifested all of the key traits of PGPR. Some of these strains were capable of biosynthesizing indolic compounds in the presence of L-tryptophan. Indole 3-acetic acid was produced by Azotobacter sp. AJ 1.1, Pseudomonas sp. PJ 1.2, and tree strains belonging to the Pantoea species: DKB 64, DKB 65, and DKB 70. Seven strains tested are very promising for the production of siderophores, iron chelating compounds. These included the strains of Azotobacter sp. AJ 1.1, Pseudomonas sp. PJ 1.2, and all those belonging to Bacillus sp. Among the tested strains, seven showed the ability to convert insoluble inorganic phosphorus (P) compounds, such as tricalcium phosphate, to bioavailable forms (Azotobacter sp. AJ 1.1, Pseudomonas sp. PJ 1.2, and all those belonging to Pantoea sp.). Ten strains expressed the ACC deaminase (ACCD) activity at levels ranging from 62.5 to 8306 nmol α-KB·mg protein⁻¹·h⁻¹. The highest ACCD activity (8306.25 nmol α-KB·mg protein⁻¹·h⁻¹) was exhibited by Azotobacter sp. AJ 1.1, followed by Pseudomonas sp. PJ 1.2 (6287.5 nmol α-KB·mg protein⁻¹·h⁻¹), two strains of Pantoea sp.: DKB 65 (4757 nmol α-KB·mg protein⁻¹·h⁻¹) and DKB 68 (3862.25 nmol α-KB·mg protein⁻¹·h⁻¹). The ACCD activity of the Bacillus sp. strains was expressed at levels ranging from 2946.75 (DKB 58) to 853 (DKB 26) nmol α-KB·mg protein⁻¹·h⁻¹. Two strains belonging to Bacillus sp. (DLGB 2 and DLGB 3) showed no ACCD activity (Table 1).

### Table 1. Some of the key traits of plant growth-promoting bacteria strains.

| Bacteria Strain | IAA Production | Siderophore Production | Phosphate Solubilization | ACCD Activity (nmol α-KB·mg Protein⁻¹·h⁻¹) |
|-----------------|----------------|------------------------|--------------------------|------------------------------------------|
| AJ 1.1          | +              | +                      | +                        | 8306.25 ± 114.2                         |
| PJ 1.2          | +              | +                      | +                        | 6287.5 ± 122.4                          |
| DLGB 2          | -              | +                      | -                        | nd                                       |
| DLGB 3          | -              | +                      | -                        | nd                                       |
| DKB 26          | -              | -                      | +                        | 853.0 ± 25.7                            |
| DKB 58          | -              | +                      | -                        | 2946.75 ± 108.6                         |
| DKB 84          | -              | +                      | -                        | 2742.0 ± 46.0                           |
| DKB 64          | +              | -                      | +                        | 3862.25 ± 34.5                          |
| DKB 65          | +              | -                      | +                        | 4757.0 ± 75.7                           |
| DKB 70          | +              | -                      | +                        | 62.5 ± 29.2                             |
| DKB 63          | -              | -                      | +                        | 107.0 ± 17.3                            |
| DKB 68          | -              | -                      | +                        | 122.75 ± 23.7                           |

-: no activity; +, activity; nd, not detected.

2.2. Bacterial Counts in Substrate

In the present study, in the case of optimum moisture content, the lowest numbers of bacteria were observed in the control variants, i.e., K0 (3.2 × 10⁵ CFU/g of substrate) and KMg with magnesium sulfate (7.2 × 10⁵ CFU/g of substrate). Only one strain, Pantoea sp. DKB 63, caused a reduction in the number of bacteria in relation to KMg (to the level of 1.2 × 10⁵ CFU/g of substrate), while the remaining strains caused an increase in the number of bacteria in the soil. The highest numbers of bacteria were observed after inoculation with Pseudomonas sp. PJ 1.2 (2.1 × 10⁷ CFU/g of substrate) and Azotobacter AJ 1.1 (1.3 × 10⁷ CFU/g of substrate). In the case of moisture deficit in the substrate, a negative correlation was observed for all the variants at the level of −0.46, which was manifested in a considerable reduction in the total number of bacteria in the soil. The lowest values of CFU per g of substrate were recorded for the controls K0 (8.5 × 10⁴) and KMg (1.3 × 10⁵). After bacterial inoculation, the highest numbers of bacteria were recorded for the strains of Pseudomonas sp. PJ 1.2 (8.2 × 10⁵ CFU/g of substrate), Pantoea sp. DKB
considerable reduction in the total number of bacteria in the soil. The lowest values of CFU per g of substrate were recorded for the controls K0 (8.5 × 10^3) and KMg (1.3 × 10^4).

After bacterial inoculation, the highest numbers of bacteria were recorded for the strains of Pseudomonas sp. PJ 1.2 (8.2 × 10^5 CFU/g of substrate), Pantoea sp. DKB 64 (3.8 × 10^5 CFU/g of substrate), and Bacillus sp. DKB 58 (3.3 × 10^5 CFU/g of substrate) (Figure 1).

![Figure 1. Bacterial counts in growth substrate after inoculation with rhizosphere bacteria under different levels of substrate moisture.](image)

2.3. Chlorophyll Fluorescence

The study analyzed the frequency of statistically significant differences in the values of the determined parameters of chlorophyll “a” fluorescence in strawberry, between the control variants (K0 and KMg) and the variants differing in the bacterial strains used for inoculation, regardless of the substrate moisture level. The analysis revealed four inoculation strains that accounted for 56% of the variability of chlorophyll “a” fluorescence indices; they were: Bacillus sp. DKB 58, Pantoea sp. DKB 64, Pantoea sp. DKB 65, and Pantoea sp. DKB 68. It should be noted that after including the next three strains—i.e., Bacillus sp. DKB 84, Pantoea sp. DKB 70, and Bacillus sp. DKB 26—the seven strains together accounted for 85% of the variability of the determined indices of chlorophyll “a” fluorescence, regardless of the substrate moisture level (Figure 2).
The frequency of statistically significant changes in the indices of chlorophyll “a” fluorescence in strawberry as a result of inoculation with rhizosphere bacteria, between optimal soil moisture and water deficit conditions.

The hierarchical cluster analysis revealed three important clusters: cluster I and cluster II represented the similarity of the variability of chlorophyll “a” fluorescence indices under the conditions of water deficit in the substrate, and cluster III was a complex cluster encompassing the variability under optimal moisture levels. Cluster I included strains whose variability coincided with the changes in the control samples K0 and KMg. Cluster II, which was mainly responsible for the variability of these traits caused by different moisture levels, included the strains: Pantoea sp. DKB 64, Pantoea sp. DKB 68, Pantoea sp. DKB 70, Bacillus sp. DKB 58, Pantoea sp. DKB 65, and Bacillus sp. DKB 26. Cluster III showed the dependence of the variability of the effect of the inoculation strains on the magnitude of the determined indices of chlorophyll “a” fluorescence in a manner dependent on K0 and KMg. The most important feature observed in the experiment is the total distinctiveness in terms of the tested fluorescence indices between the optimal soil moisture and water deficit (Figure 3).

The results of the descriptive statistics of the conducted experiment are presented in Tables 2 and 3 and Figures 4–10. The results of the analysis of variance are shown in Table 4. The F₀ coefficient varied in the range from 193 to 376 in the conditions of optimal moisture, reaching a CV of 9.5%, while in the conditions of water deficit, the coefficient was between 159 and 373 and CV = 11%. The lowest values were recorded when inoculated with the Bacillus sp. DLGB 2 (OM) and Pantoea sp. DKB 63 (WD) strains, and the highest when inoculated with the Bacillus sp. DLGB 2 (OM) and Pantoea sp. DKB 63 (WD) strains (Table 2, Figure 4).

Figure 2. Frequency of statistically significant changes in the indices of chlorophyll “a” fluorescence in strawberry as a result of inoculation with rhizosphere bacteria, between optimal soil moisture and water deficit conditions.

| Variant | Descriptive Statistics | TₐM | Area |
|---------|------------------------|-----|------|
|         |                        | OM  | WD   |
| K0      | Mean ± SD              |     |      |
|         | Range                  |     |      |
|         | CV                     |     |      |
|         | 837.5 ± 16.06          | 858.33 ± 11.86 | 69,490.33 ± 28 | 61,215.92 ± 19.76 | 230.63 ± 10.61 | 232.38 ± 14.6 |
|         | 400–900                | 500–900 | 29,086–98,646 | 30,210–89,071 | 202–312 | 202–371 |
| KMg     | Mean ± SD              |     |      |
|         | Range                  |     |      |
|         | CV                     |     |      |
|         | 891.67 ± 4.58          | 879.17 ± 6.69 | 72,806.5 ± 19.45 | 66,549.96 ± 18.08 | 222.08 ± 5.84 | 224.33 ± 12.27 |
|         | 700–900                | 700–900 | 53,216–10,2457 | 29,279–89,552 | 202–255 | 202–343 |
| AJ 1.1  | Mean ± SD              |     |      |
|         | Range                  |     |      |
|         | CV                     |     |      |
|         | 866.67 ± 10.24         | 875 ± 5.17 | 70,891.58 ± 23.05 | 63,561 ± 21.7 | 234.08 ± 10.7 | 226.42 ± 6.3 |
|         | 600–900                | 800–900 | 32,248–86,547 | 41,521–81,726 | 216–308 | 205–253 |

Table 2. Descriptive statistics results.
Table 2. Cont.

| Variant | Descriptive Statistics | Trm | Area | F0 |
|---------|------------------------|-----|------|----|
| PJ 1.2  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DLGB 2  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 26  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 58  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 84  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 64  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 65  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 70  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 63  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |
| DKB 68  | Mean ± SD              |     |      |    |
|         | Range CV               |     |      |    |

Table 3. Descriptive statistics results.

| Variant | Descriptive Statistics | F0 | Fv | Fv/F0 | PI |
|---------|------------------------|----|----|--------|----|
| K0      | Mean ± SD              |     |    |        |    |
|         | Range CV               |     |    |        |    |
| KMg     | Mean ± SD              |     |    |        |    |
|         | Range CV               |     |    |        |    |
| AJ 1.1  | Mean ± SD              |     |    |        |    |
|         | Range CV               |     |    |        |    |
| PJ 1.2  | Mean ± SD              |     |    |        |    |
|         | Range CV               |     |    |        |    |
| DLGB 2  | Mean ± SD              |     |    |        |    |
|         | Range CV               |     |    |        |    |
| DKB 3   | Mean ± SD              |     |    |        |    |
|         | Range CV               |     |    |        |    |
| DKB 26  | Mean ± SD              |     |    |        |    |
|         | Range CV               |     |    |        |    |
### Table 3. Cont.

| Variant  | Descriptive Statistics | F<sub>M</sub> | F<sub>v</sub> | F<sub>v/F<sub>m</sub></sub> | PI |
|----------|------------------------|--------------|--------------|-----------------|----|
|          | OM ± SD                | WD ± SD      | OM ± SD      | WD ± SD         | OM ± SD |
|          | Mean ± SD              | Mean ± SD    | Mean ± SD    | Mean ± SD       | Mean ± SD |
|          | Range                  | Range        | Range        | Range           | Range |
| DKB 58   | 1317.5 ± 7.21          | 1263.33 ± 6.15 | 1083.42 ± 9.66 | 1045.58 ± 7.36 | 0.82 ± 3.22 | 0.83 ± 1.39 | 9.95 ± 34.12 | 11.81 ± 16.31 |
|          | 1182-1461              | 1122-1393    | 937-1227     | 903-1176        | 0.75-0.85 | 0.81-0.84 | 3.03-14.78 | 7.56-14.65 |
| DKB 84   | 1321.5 ± 6.86          | 1164 ± 12.14 | 1087.42 ± 9.99 | 941.92 ± 15.53 | 0.82 ± 4.27 | 0.81 ± 3.85 | 10.06 ± 34.33 | 10.3 ± 39.23 |
|          | 1116-1443              | 912-1376     | 867-1212     | 674-1156        | 0.72-0.84 | 0.74-0.85 | 1.94-15.89 | 3.03-16.52 |
| DKB 64   | 1276.42 ± 10.22        | 1293.67 ± 4.72 | 1043.17 ± 13.13 | 1081.75 ± 5.78 | 0.82 ± 3.53 | 0.82 ± 1.33 | 8.66 ± 37.17 | 15.01 ± 16.9 |
|          | 1038-1469              | 1177-1378    | 813-1238     | 961-1163        | 0.77-0.85 | 0.82-0.85 | 4.5-14.4 | 10.07-19.1 |
| DKB 65   | 1271.58 ± 7.67         | 1272.17 ± 5.07 | 1056.5 ± 9.28 | 1052.83 ± 6.08 | 0.83 ± 1.72 | 0.83 ± 1.27 | 12.49 ± 18.47 | 11.49 ± 24 |
|          | 1122-1395              | 1163-1348    | 916-1182     | 942-1128        | 0.81-0.85 | 0.81-0.84 | 8.56-16.52 | 6.33-15 |
| DKB 70   | 1271.82 ± 9.05         | 1219.91 ± 10.61 | 1037.18 ± 11.72 | 1006.45 ± 12.43 | 0.81 ± 3.24 | 0.82 ± 2.06 | 8.63 ± 28.29 | 13.16 ± 21.64 |
|          | 1059-1485              | 952-1398     | 837-1193     | 758-1176        | 0.77-0.84 | 0.8-0.85 | 5.4-12.84 | 8.13-18.44 |
| DKB 63   | 1294.25 ± 9.57         | 1189.25 ± 16.63 | 1064.42 ± 12.34 | 963.58 ± 21.53 | 0.82 ± 4 | 0.8 ± 3.9 | 10.66 ± 36.75 | 11.94 ± 43.75 |
|          | 1070-1458              | 779-1426     | 844-1234     | 554-1198        | 0.74-0.85 | 0.6-0.84 | 3.23-15.69 | 0.27-18.92 |
| DKB 68   | 1276.75 ± 10.35        | 1183 ± 10.26 | 1048.58 ± 12.96 | 958.83 ± 14.72 | 0.82 ± 3.03 | 0.81 ± 5.86 | 10.4 ± 29.29 | 11.56 ± 49.21 |
|          | 994-1449               | 970-1342     | 756-1221     | 646-1130        | 0.76-0.85 | 0.67-0.84 | 4.53-13.88 | 1.02-23.96 |

**Figure 3.** Cluster analysis showing the similarities of the variability of chlorophyll “a” fluorescence indices, depending on the different substrate moisture levels and inoculation with rhizosphere bacteria, where: OM—optimal moisture, WD—water deficit.
Figure 4. $F_0$ in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.

Figure 5. $F_M$ in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.
Figure 6. $F_V$ in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.

Figure 7. $F_V/F_M$ in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.
Figure 7. FV/FM in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.

Figure 8. TFM in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.

Figure 9. PI in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.

Figure 10. 'Area' parameter in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.
Figure 9. PI in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.

Figure 10. ‘Area’ parameter in the leaves of strawberry plants inoculated with rhizosphere bacteria growing under different substrate moisture levels, where: OM—optimal moisture, WD—water deficit.

Table 4. ANOVA results.

| Variable | Sum of Squares | df | Mean Square Error | F     | p      |
|----------|---------------|----|-------------------|-------|--------|
| TFM      | 18,984        | 29 | 5539              | 3.42725 | 0.064900 |
| Area     | 958,826,171   | 29 | 200,351,660       | 4.78572 | 0.029302 |
| F0       | 6257          | 29 | 600               | 10.43421 | 0.001344 |
| FM       | 141,911       | 29 | 13,807            | 10.27838 | 0.001459 |
| FV       | 88,573        | 29 | 14,966            | 5.91849  | 0.015441 |
| FV/FM    | 0.00007       | 29 | 0.0009            | 0.08489  | 0.770939 |
| PI       | 220           | 29 | 13               | 16.75891 | 0.000052 |

The FM coefficient averaged 1258 and varied in the range from 984 to 1487 under optimal moisture conditions, reaching a CV of 6.5%, while under water deficiency conditions, the coefficient was between 779 and 1464 and CV = 7%. The lowest values were recorded in the control K0 (OM) and when inoculated with the Pantoea sp. DKB 63 (WD) strain, and the highest when inoculated with the Pseudomonas sp. PJ 1.2 (OM) strain and KMg (WD) (Table 4, Figure 5).

The FV coefficient averaged 1032 and varied from 736 to 1259 under optimal moisture conditions, reaching a CV of 7.5%, while under water deficit, the coefficient was between 554 and 1237 and CV = 9.8%. The lowest values were recorded in the control K0 (OM) and when inoculated with the Pantoea sp. DKB 63 (WD) strain, and the highest when inoculated with the Pseudomonas sp. PJ 1.2 (OM) strain and KMg (WD) (Table 4, Figure 6).

The FV/FM ratio averaged 0.82 and fluctuated in the range from 0.64 to 0.85 under optimal moisture conditions, reaching a CV of 4.1%, while under water deficit, the ratio was between 0.60 and 0.85 with CV = 3.6%. The lowest values were recorded at inoculation with the Bacillus sp. DLGB 2 (OM) and Pantoea sp. DKB 63 (WD) strains, and the highest...
in inoculation with the *Bacillus* sp. DLGB 3 (OM) and *Pantoea* sp. DKB 70 (WD) strains (Table 4, Figure 7).

The PI coefficient was on average 10.77 and varied in the range from 0.51 to 17.72 under optimal moisture conditions, reaching a CV of 34%, while under water deficit, the coefficient was between 0.27 and 23.97 with CV = 29.8%. The lowest values were recorded during inoculation with the strains *Bacillus* sp. DLGB 2 (OM) and *Pantoea* sp. DKB 63 (WD), and the highest when inoculated with *Bacillus* sp. DLGB 3 (OM) and *Pantoea* sp. DKB 68 (WD) (Table 2, Figure 9).

The Area coefficient averaged 69.568 and varied in the range from 27,371 to 102,457 under optimal moisture conditions, reaching a CV of 17%, while under water deficit, the coefficient was between 17,269 and 107,411, CV = 19% The lowest values were recorded when inoculating with the *Bacillus* sp. DLGB 3 strains (OM) and *Pantoea* sp. DKB 63 (WD), and the highest at KMg (OM) and *Pantoea* sp. DKB 70 (WD) (Table 2, Figure 10).

3. Discussion

Members of the *Bacillus*, *Pseudomonas*, and *Azotobacter* genera of bacteria have been extensively reported as plant growth enhancers [41–45]. Those of the genus *Pantoea*, although usually known as plant pathogens, have been reported in some studies to include strains with plant growth-promoting capabilities [32,46,47]. Indole-3-acetic acid (IAA), a plant hormone, is a natural auxin produced by rhizobacteria; as one of the phytohormones, IAA can act as a reciprocal signaling molecule in microbe-plant interactions [48]. Siderophore-producing PGPRs increased the Fe(III) ion supply to plants in the rhizosphere and are, therefore, known to enhance plant growth and crop productivity [49]. The ability to produce a clear zone around the bacterial colony implies that the bacteria can solubilize mineral phosphorus in the rhizosphere [50]. Plant growth-promoting rhizobacteria which possess the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase facilitate plant growth and development by decreasing ethylene levels, inducing a reduction in drought stress in plants [51]. The production of key traits for PGPR strains was found among many representatives of the bacteria used in this work and they were also tested in the context of drought [52–55].

There are a number of reports on the mitigation of adverse effects of drought by the addition of PGP microbes [56,57]. The most often used for this purpose are strains of the genus *Bacillus* [27,58,59], with those of *Pantoea* [60] and *Azotobacter* [61] used to a lesser extent.

There are not many reports on the changes in the numbers of microorganisms under drought conditions. For example, culture-based methods (intact grassland monoliths from natural habitats) have indicated that microbial physiological response was modulated by moisture content. The highest numbers of bacteria were observed with wetted treatments consistently being over 10^8 CFU/g of substrate, and there was up to a 40-fold reduction in CFU in dried treatments, compared with the continually wetted treatments [62]. Similar observations were noted by Omar et al. [63], where the decrease in the numbers of bacteria, after they had been subjected to drought stress, varied according to the rice genotype from 9.3% to 20%. When comparing the survival of *Azospirillum* strains under stressful conditions in maize cultivation, Ilyas et al. [64] observed a decrease in the number of these bacteria by 40%.

In this study, the smallest decrease in the number of bacteria in the case of moisture deficit, compared with optimum moisture content, was observed following inoculations with the strains *Pantoea* sp. DKB 65 (approx. 5 times), *Bacillus* sp. DKB 58 (approx. 11 times) and *Bacillus* sp. DLGB 2 (approx. 13 times).

Chlorophyll fluorescence can be considered the basic indicator for the analysis of the relationship between photosynthesis and plant growth environment. It is used, inter alia, in studies on the response of various plant species to stressors [65–67]. Under the influence of water deficiency, the probability of PSII damage increases, manifested by a reduced
photosynthetic efficiency and an increase in the dissipation of absorbed energy in the form of non-photochemical quenching [68].

The study did not show any influence of the experimental factors or their interactions on the values of the parameters $F_0$ and $T_{FM}$. The $F_0$ index indicates the amount of loss in the excitation energy during its transmission from the antennas to the PSII active center. $T_{FM}$ is the time to peak fluorescence. In plants growing under the conditions of water deficiency, the study found a decrease in the parameters $F_M$, $F_V$, $F_V/F_M$, and also PI and Area, which confirmed the presence of the state of stress. This is indicative of a significant impact of drought on the state of the photosynthetic apparatus of the tested plant species, a lower quantum yield of PSII, the inability to reduce all electron acceptors, and the occurrence of energy losses in the form of heat. According to Xu [69] and Angelini et al. [70], the ratio $F_V/F_M$ is considered a reliable indicator of the photochemical activity of the photosynthetic apparatus, which determines the potential efficiency of PSII. When this ratio becomes lower, it proves that the plant has been exposed to a stressor. A reduction in the $F_V/F_M$ value due to drought, which was demonstrated in our study, has also been observed in other species such as: *Lycopersicon esculentum* Mill. [71], *Viburnum tinus* L. [72], and *Vigna inguiculata* L. Walp. [73]; in the case of the latter—as a result of prolonged water deficiency stress. There are also reports of the relatively low sensitivity of the $F_V/F_M$ ratio to water deficiency, which has been found in various species: *Phaseolus vulgaris* L. [74], *Glycine max.* (L.) Merr [75], *Secale cereale* L. [76], and also *Fragaria × ananassa* Duch. [77]. The PI index is considered a reliable parameter for assessing the tolerance of plants to abiotic stresses and is an indicator of the efficiency of the PSII system [78]. Our study showed a significant decrease in the value of this index by 10.6% due to water deficiency. Similar results of research on the impact of drought stress on the PI parameter in rye had been reported by Czyczyl-Myszynski and Myśków [76].

There is evidence that by inducing various mechanisms, such as the production of phytohormones (IAA, cytokinins, ABA), the production of bacterial exopolysaccharides (EPS), and the synthesis of the ACC deaminase enzyme, rhizospheric microorganisms can promote plant growth under drought stress [79,80]. In the present study, the inoculation with the tested strains of rhizospheric bacteria did not affect the value of the parameters $F_M$, $F_V$, and $F_V/F_M$, neither under the conditions of drought nor under optimal substrate moisture (Figures 5–7). Barnawal et al. [81], however, had shown, under water deficit, a beneficial effect of the inoculation of wheat with the *Bacillus subtilis* LDR2 strain on the efficiency of the PSII system, which was manifested by an increase in the $F_V/F_M$ ratio. Similarly, Khan et al. [82] had shown an increase in $F_V/F_M$ as a result of using a consortium of the bacteria *Bacillus subtilis*, *Bacillus thuringiensis*, and *Bacillus megaterium* in two varieties of *Cicer arietinum* L. contrasting for drought tolerance, growing under water deficit. This increase was particularly evident in the drought-sensitive variety.

In the case of the plants growing under water deficit, the present study showed an increase, in comparison with the control, in the PSII vitality index due to inoculation with the *Bacillus* sp. strains, DLGB2 and DKB26, as well as the *Pantoea* sp. strains, DKB63, DKB70, DKB68, and DKB64. There was also evidence of a beneficial effect of inoculation with the *Pantoea* sp. strains, DKB70, DKB68, and DKB65 on the increase, relative to the control, in the value of the Area parameter in the strawberry plants growing under the conditions of water deficit in the substrate. This may indicate an increase in the efficiency of electron transport from the reaction centers to the plastoquinones. A synergistic effect of the *Pseudomonas* strains in the production of ACC deaminase, auxin synthesis, the ability of mineral phosphate solubilizing, and the production of siderophores, has been found to significantly improve the yield-related traits of sweetcorn under the limited availability of irrigation water. Moreover, there was an increase in the $F_V/F_M$ index and a decrease in the $F_0$ index after inoculation with bacteria [83].
4. Materials and Methods

4.1. Location of the Experiment, Plant Material, and Growth Conditions

The experiment was conducted in a greenhouse, located at the West Pomeranian University of Technology in Szczecin (53°25’ N, 14°32’ E, 25 m a.s.l., sub-zone 7a USDA). On 5 October 2020, plantlets of the strawberry cultivar Polka (Strawberry plant nursery J.G. Mendyk, Koronowo, Poland) were planted. Strawberry cv. Polka is one of the tastiest medium-late varieties of strawberries. The fruits are medium size, spherical, heart-shaped, or broad-conical-shaped. They have uniformly red to dark red skin with a slight gloss. The plants are moderately strongly growing. ‘Polka’ is resistant to frost, leaf scorch disease, as well as powdery mildew, and are susceptible to common gray mold. Plantlets (BBCH13) were planted individually into black round PVC pots with a diameter of 19 cm, and a capacity of 3.0 dm³, filled with peat substrate (Substral Osmocote, Evergreen Garden Care Poland Sp. Z o. o.), and mixed with perlite (at 15:1)—Figure 11. The substrate (pH 6.2) was enriched with a 2 g dm⁻³ mixture of Osmocote NPK 15-09-09 and Plant Starter NPK 10-52-10. No additional fertilization was applied during plant vegetative growth. The plants were cultivated under natural day/night conditions, 8h:16h day: night, without artificial lighting, at 17–20 °C.

Figure 11. The plantlet of the strawberry cv. Polka.

4.2. Experimental Factors

A two-factor experiment was performed in a complete randomization design with four replications, each represented by a single plant. The first experimental factor was the inoculation of plant roots with rhizospheric bacteria. The inoculum was applied to the growth substrate near the root system, in the amount of 40 cm³/plant, in the amount of 40 cm³/plant, +

- K0—plants not inoculated with rhizospheric bacteria,
- KMg—application of MgSO₄ nutrient solution without bacteria to the growth substrate in the amount of 40 cm³/plant, +
- DLGB 2—inoculation with Bacillus sp. strain DLGB 2,
- DLGB 3—inoculation with Bacillus sp. strain DLGB 3,
- DKB 26—inoculation with Bacillus sp. strain DKB 26,
- DKB 58—inoculation with Bacillus sp. strain DKB 58,
- DKB 84—inoculation with Bacillus sp. strain DKB 84,
- DKB 63—inoculation with Pantoea sp. strain DKB 63,
- DKB 64—inoculation with Pantoea sp. strain DKB 64,
- DKB 65—inoculation with Pantoea sp. strain DKB 65,
with Whatman No. 1 filter paper saturated with Salkowski’s reagent for 30 min. at 28 °C (TSA, Oxoid). After incubation at 28 °C, a pink zone appeared around the IAA-producing colonies.

sodium chloride. After homogenization for 1 h, this solution was decimally diluted (10⁻⁴ to 10⁻⁶), and 100 µL aliquots of the resulting solutions were plated on Tryptone Soya Agar (TSA, Oxoid). After incubation at 28 °C for 3 days, the colony forming units (CFU) were quantified of indole-3-acetic acid production was performed with Salkowski’s reagent [87]. Bacterial cultures were grown on minimal DF solid medium [88] supplemented with tryptophan (500 µg·mL⁻¹) as the precursor of IAA. The plates were covered with Whatman No. 1 filter paper saturated with Salkowski’s reagent for 30 min. at 28 °C. A pink zone appeared around the IAA-producing colonies.

The mobilization of P from insoluble phosphate was detected by the formation of a transparent halo zone surrounding bacterial colonies on the Pikovskaya medium containing tricalcium phosphate, after 5 days at 28 °C [84]. Siderophore production was detected by the production of an orange halo zone on a standard Chrome Azurol-S (CAS) agar plate after 5 days at 28 °C [85,86].

The quantification of ACC deaminase activity was determined by a modified method that measures the amount of α-ketobutyrate (α-KB) when the ACC deaminase enzyme cleaves ACC. The bacterial strains were propagated in a minimal DF medium with 5 mM ACC. The calibration curve was formed on α-ketobutyrate. The ACCD activity was expressed as nM of α-KB·mg protein⁻¹·h⁻¹ [89,90].

4.4. Bacterial Counts in Substrate

Two grams of each substrate sample was added to 18 mL of 0.9% (w/v) solution of sodium chloride. After homogenization for 1 h, this solution was decimally diluted (10⁻² to 10⁻⁶), and 100 µL aliquots of the resulting solutions were plated on Tryptone Soya Agar (TSA, Oxoid). After incubation at 28 °C for 3 days, the colony forming units (CFU) were determined using soil contact tensiometers.

---

**Figure 12.** Strawberry cv. Polka at the time of inoculation.

The Bacillus sp. and Pantoea sp. inocula came from the Department of Microbiology and Rhizosphere, the National Institute of Horticultural Research in Skierniewice (Poland); the strains of Azotobacter sp. and Pseudomonas sp. came from the Institute of Marine and Environmental Sciences of the University of Szczecin (Poland).

The second experimental factor was the different moisture content of the growth substrate. The water potential was maintained at −10 to −15 kPa under control conditions (optimal soil moisture—variant OM), and at −40 to −45 kPa under conditions of water deficit in the substrate (variant WD). The substrate moisture levels were varied from 6 weeks after inoculation. The substrate moisture was determined using soil contact tensiometers.

4.3. Bioassays for Plant Growth Promoting Traits

The mobilization of P from insoluble phosphate was detected by the formation of a transparent halo zone surrounding bacterial colonies on the Pikovskaya medium containing tricalcium phosphate, after 5 days at 28 °C [84]. Siderophore production was detected by the production of an orange halo zone on a standard Chrome Azurol-S (CAS) agar plate after 5 days at 28 °C [85,86].

The quantification of indole-3-acetic acid production was performed with Salkowski’s reagent [87]. Bacterial cultures were grown on minimal DF solid medium [88] supplemented with tryptophan (500 µg·mL⁻¹) as the precursor of IAA. The plates were covered with Whatman No. 1 filter paper saturated with Salkowski’s reagent for 30 min. at 28 °C. A pink zone appeared around the IAA-producing colonies.

The mobilization of P from insoluble phosphate was detected by the formation of a transparent halo zone surrounding bacterial colonies on the Pikovskaya medium containing tricalcium phosphate, after 5 days at 28 °C [84]. Siderophore production was detected by the production of an orange halo zone on a standard Chrome Azurol-S (CAS) agar plate after 5 days at 28 °C [85,86].

The quantification of indole-3-acetic acid production was performed with Salkowski’s reagent [87]. Bacterial cultures were grown on minimal DF solid medium [88] supplemented with tryptophan (500 µg·mL⁻¹) as the precursor of IAA. The plates were covered with Whatman No. 1 filter paper saturated with Salkowski’s reagent for 30 min. at 28 °C. A pink zone appeared around the IAA-producing colonies.

The second experimental factor was the different moisture content of the growth substrate. The water potential was maintained at −10 to −15 kPa under control conditions (optimal soil moisture—variant OM), and at −40 to −45 kPa under conditions of water deficit in the substrate (variant WD). The substrate moisture levels were varied from 6 weeks after inoculation. The substrate moisture was determined using soil contact tensiometers.

4.3. Bioassays for Plant Growth Promoting Traits

The mobilization of P from insoluble phosphate was detected by the formation of a transparent halo zone surrounding bacterial colonies on the Pikovskaya medium containing tricalcium phosphate, after 5 days at 28 °C [84]. Siderophore production was detected by the production of an orange halo zone on a standard Chrome Azurol-S (CAS) agar plate after 5 days at 28 °C [85,86].

The quantification of indole-3-acetic acid production was performed with Salkowski’s reagent [87]. Bacterial cultures were grown on minimal DF solid medium [88] supplemented with tryptophan (500 µg·mL⁻¹) as the precursor of IAA. The plates were covered with Whatman No. 1 filter paper saturated with Salkowski’s reagent for 30 min. at 28 °C. A pink zone appeared around the IAA-producing colonies.

The second experimental factor was the different moisture content of the growth substrate. The water potential was maintained at −10 to −15 kPa under control conditions (optimal soil moisture—variant OM), and at −40 to −45 kPa under conditions of water deficit in the substrate (variant WD). The substrate moisture levels were varied from 6 weeks after inoculation. The substrate moisture was determined using soil contact tensiometers.
counted. The bacterial counts were performed on substrate samples taken from each pot on 12 April 2021, 18 weeks after the inoculation of the plants.

4.5. Chlorophyll “a” Fluorescence

The measurements of direct chlorophyll fluorescence were recorded using a Handy PEA (Handy Plant Efficiency Analyzer) spectrofluorometer (Hansatech Instruments Ltd., King’s Lynn, Norfolk, UK), based on the standard apparatus procedure (3 × 650 nm LEDs, maximum actinic light intensity 3500 µmol·m⁻²·s⁻¹, duration of the light pulse 1s). The leaves were shaded for 20 min. prior to the measurement with a leaf clip (4 mm in diameter). The following parameters of chlorophyll fluorescence induction were measured and calculated using the spectrofluorometer: the index of initial fluorescence excitation energy loss in power antennas (F₀); maximum fluorescence after the reduction in acceptors in PSII and after dark adaptation (Fₚₘ); variable fluorescence, determined after dark adaptation, a parameter dependent on the maximum quantum yield of PSII (Fᵥ = Fₚₘ − F₀); maximum potential photochemical reaction efficiency in PSII determined after dark adaptation and after the reduction in acceptors in PS II (Fᵥ/Fₚₘ); the time of fluorescence increase to the value of Fₚₘ (Tₚₘ); PSII vitality index for the overall viability of this system (PI); the surface area above the chlorophyll fluorescence curve and between the F₀ and Fₚₘ points proportional to the size of the reduced plastoquinone acceptors in PS II (Area) [91]. The measurements of chlorophyll fluorescence parameters were taken 18 weeks after the inoculation of the plants, on healthy, fully grown leaves of each plant.

4.6. Statistical Methods

The results of the tests were subjected to bivariate analysis of variance (ANOVA), and Tukey’s HDS post hoc test was performed. Additionally, in order to determine the occurrence of the variability of the determined traits depending on the experimental factors used, a cluster analysis was carried out using Ward’s method for linkage and the square of the Euclidean distance as a measure of distance [92]. The significance of the clusters was determined using the Sneath graded criterion [93]. The above statistical calculations were performed using Statistica 13.1PL (Cracow, Poland, StatSoft Poland). A Monte Carlo simulation with R Studio software (Boston, USA, RStudio PBA) was performed to confirm the obtained results [94].

5. Conclusions

The presented studies in this paper on strawberry plants showed that the greatest usefulness for characterizing water stress was demonstrated by Fₚₘ, Fᵥ, Fᵥ/Fₚₘ, PI, and Area. Based on the assessment of the condition of the photosynthetic apparatus and the analysis of chlorophyll “a” fluorescence indices, the most favorable effects on strawberry plants under water deficit had the Bacillus sp. strains, DLGB2 and DKB26 and the Pantoea sp. strains, DKB63, DKB70, DKB68, DKB64, and DKB65. In the case of inoculation with the Pantoea sp. strain, DKB65 and Bacillus sp. strain, DKB64, was one of those that produced, after inoculation under water deficit, the highest number of bacteria. The recently ongoing climatic changes force us to look for sustainable and effective solutions to alleviate the water deficit stress in cultivated plants. Therefore, it is justified and necessary to conduct further, much wider research on the most promising beneficial microorganisms that favorably affect plants under stress conditions and alleviate the negative effects of water stress.

Author Contributions: Conceptualization, D.P., G.M. and M.M.; methodology, D.P., G.M., M.M., A.K., L.S.-P. and T.M.; validation, D.P., G.M., M.M., A.K. and T.M.; formal analysis, D.P., G.M., M.M., A.K. and T.M.; investigation, D.P., G.M., M.M. and A.K.; data curation, D.P., G.M., M.M., A.K. and T.M.; writing—original draft preparation, D.P., G.M., M.M., A.K., L.S.-P. and T.M.; writing—review and editing, D.P., G.M., M.M., A.K., L.S.-P. and T.M.; visualization, D.P. and T.M.; supervision, G.M.; All authors have read and agreed to the published version of the manuscript.
Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data stored on the PTBD BIODATA server and in the public repository: https://github.com/PTBD-BIODATA/Databases/blob/main/Fluoro%20DB.csv.

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

References

1. Golldack, D.; Li, C.; Mohan, H.; Probst, N. Nerve growth factor and mental stress in plants: Unraveling the signaling networks. Front. Plant Sci. 2014, 5, 151. [CrossRef] [PubMed]

2. Kumar, A.; Verma, J.P. Does plant-microbe interaction confer stress tolerance in plants? A review? Microbiol. Res. 2018, 207, 41–52. [CrossRef] [PubMed]

3. Hura, T.; Grzesiak, S.; Hura, K.; Thielt, E.; Tokarz, K.; Wędzony, M. Physiological and biological tools useful in drought-tolerance detection in genotypes of winter triticale: Accumulation of ferulic acid correlates with drought tolerance. Ann. Bot. 2007, 100, 767. [CrossRef]

4. Zhou, Y.; Lam, H.M.; Zhang, J. Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. J. Exp. Bot. 2007, 58, 1207–1217. [CrossRef]

5. Kalaji, M.H.; Pietkiewicz, S. Some physiological indices to be exploited as a crucial tool in plant breeding. Plant Breed. Seed Sci. 2004, 49, 19–39.

6. Philippot, L.; Raaijmakers, J.M.; Lemanceau, P.; van Der Putten, W.H. Going back to the roots: The microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 2013, 11, 789–799. [CrossRef]

7. Berg, G.; Grube, M.; Schloter, M.; Smalla, K. Unraveling the plant microbiome: Looking back and future perspectives. Front. Microbiol. 2014, 5, 148. [CrossRef]

8. Mikiciuk, G.; Sas-Paszt, L.; Mikiciuk, M.; Derkowska, E.; Trzcinski, P.; Gluszek, S.; Lisick, A.; Wera-Bryl, S.; Rudnica, J. Mycorrhizal frequency, physiological parameters, and yield of strawberry plants inoculated with endomycorrhizal fungi and the rhizosphere bacteria. Mycorrhiza 2019, 29, 489–501. [CrossRef]

9. Yaghoubi Khandahali, 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]

10. Cohen, A.C.; Bottini, R.; Pontin, M.; Berli, F.J.; Moreno, D.; Boccanlandro, H.; Travaglia, C.N.; Piccoli, P.N. Azospirillum brasilense ameliorates the response of Arabidopsis thaliana to drought mainly via enhancement of ABA levels. Physiol Plant 2015, 153, 79–90. [CrossRef]

11. Naseem, H.; Bano, A. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. J. Plant Interaction 2014, 9, 689–701. [CrossRef]

12. Ma, Y.; Oliveira, R.S.; Freitas, H.; Zhang, C. Biochemical and molecular mechanisms of plant-microbe-metal interactions: Relevance for phytoremediation. Front. Plant Sci. 2016, 7, 918. [CrossRef]

13. Meena, H.; Ahmed, M.A.; Prakash, P. Amelioration of heat stress in wheat, Triticum aestivum by PGPR (Pseudomonas aeruginosa strain 2Cps1). Biosci. Biotech. Res. Comm 2015, 8, 171–174.

14. Chatterjee, P.; Kanagendran, A.; Samaddar, S.; Pazouki, L.; Sa, T.M.; Niinemets, Ü. Influence of Brevibacterium linens RS16 on plant photosynthesis and volatile emission characteristics and volatile emission characters on heat stress in Eucalyptus grandis. Sci. Total Environ. 2020, 700, 134453. [CrossRef]

15. Zubair, M.; Hanif, A.; Farzand, A.; Sheikh, T.M.M.; Khan, A.R.; Suleman, M.; Ayaz, M.; Gao, X. Genetic screening and expression analysis of psychrophilic Bacillus spp. reveal their potential to alleviate cold stress and modulate phytohormones in wheat. Microorganisms 2019, 7, 337. [CrossRef]

16. Azarmi, F.; Mozafari, V.; Abbaszadeh Dahaji, P.; Hamidpour, M. Biochemical, physiological and antioxidant enzymatic activity responses of pistachio seedlings treated with plant growth promoting rhizobacteria and Zn to salinity stress. Acta Physiol. Plant. 2016, 38, 21. [CrossRef]

17. Suarez, C.; Cardinale, M.; Ratering, S.; Steffens, D.; Jung, S.; Montoya, A.M.Z.; Geissler-Plaum, R.; Schnell, S. Plant growth-promoting effects of Hartmannibacter diazotrophicus on summer barley (Hordeum vulgare L.) under salt stress. Appl. Soil Ecol. 2015, 85, 23–30. [CrossRef]

18. Abdelkrim, S.; Jebra, S.H.; Saadani, O.; Abid, G.; Taamali, W.; Zemni, H.; Mennai, K.; Louati, F.; Jebra, M. In situ effects of Lathyrus sativus—PGPR to remediate and restore quality and fertility of Pb and Cd polluted soils. Ecotoxical. Environ. Saf. 2020, 192, 110260. [CrossRef]

19. Kang, S.M.; Asaf, S.; Khan, A.L.; Khan, A.; Mun, B.G.; Khan, M.A.; Gul, H.; Lee, I.J. Complete genome sequence of Pseudomonas psychrotolerans CS51, a plant growth-promoting bacterium, under heavy metal stress conditions. Microorganisms 2020, 8, 382. [CrossRef]

20. Lee, B.D.; Dutta, S.; Ryu, H.; Yoo, S.J.; Suh, D.S.; Park, K. Induction of systemic resistance in Panax ginseng against Phytophthora cactorum by native Bacillus amyloliquefaciens HK94. J. Ginseng Res. 2015, 39, 213. [CrossRef]
21. Ghazalibiglar, H.; Hampton, J.G.; de Jong, E.V.Z.; Holyoake, A. Is induced systemic resistance the mechanism for control of black rot in *Brassica oleracea* by a *Paenibacillus* sp.? *Biol. Control* 2016, 92, 195–201. [CrossRef]
22. Glick, B.R. Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica* 2012, 2012, 963401. [CrossRef]
23. Sarma, R.K.; Saikia, R. Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant Soil* 2014, 377, 111–126. [CrossRef]
24. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* 2012, 28, 1327–1350. [CrossRef]
25. Nautiyal, C.S.; Srivastava, S.; Chauhan, P.S.; Seem, K.; Mishra, A.; Sopory, S.K. Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. *Plant Physiol. Biochem.* 2013, 66, 1–9. [CrossRef]
26. Tiwari, S.; Lata, C.; Chauhan, P.S.; Nautiyal, C.S. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiol. Biochem.* 2016, 99, 108–117. [CrossRef]
27. Goswami, D.; Thakker, J.N.; Dhandhukia, P.C.; Moral, M.T. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. *Cogent Food Agric.* 2016, 2, 1127500. [CrossRef]
28. Lim, J.-H.; Kim, S.-D. Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. *Plant Pathol. J.* 2013, 29, 201–208. [CrossRef]
29. Ngumbi, E.; Kloepper, J.W. Bacterial-mediated drought tolerance: Current and future prospects. *Appl. Soil Ecol.* 2016, 105, 109–125. [CrossRef]
30. Rubin, R.L.; van Groenigen, K.J.; Hungate, B.A. Plant growth promoting rhizobacteria are more effective under drought: A meta-analysis. *Plant Soil* 2017, 416, 309–323. [CrossRef]
31. Ahmad, H.M.; Fiaz, S.; Hafeez, S.; Zahra, S.; Shah, A.N.; Gul, B.; Aziz, O.; Fakhar, A.; Rafique, M.; Chen, Y.; et al. Plant growth-promoting rhizobacteria eliminate the effect of drought stress in plants: A review. *Front. Plant Sci.* 2022, 13, 875774. [CrossRef] [PubMed]
32. Chen, C.; Xin, K.; Liu, H.; Cheng, J.; Shen, X.; Wang, Y.; Zhang, L. *Pantoea alhagi*, a novel endophytic bacterium with ability to improve growth and drought tolerance in wheat. *Sci. Rep.* 2017, 7, 41564. [CrossRef] [PubMed]
33. Uzma, M.; Ibqal, A.; Hasnain, S. Drought tolerance induction and growth promotion by indole acetic acid producing *Pseudomonas aeruginosa* in *Vigna radiata*. *PloS ONE* 2022, 17, e0262932. [CrossRef]
34. Sharma, P.; Kumawat, K.C.; Kaur, S. Plant growth promoting rhizobacteria in nutrient enrichment: Current perspectives. In *Biofortification of Food Crops*; Singh, U., Praharaj, C., Singh, S., Singh, N., Eds.; Springer: New Delhi, India, 2016; pp. 263–289.
35. Lai, S.; Chiariini, L.; Tabacchioni, S. New insights in plant-associated *Paenibacillus* species: Biocontrol and plant growth-promoting activity. In *Bacilli and Agrobiotechnology*; Islam, M., Rahman, M., Pandey, P., Jha, C., Aeron, A., Eds.; Springer: Cham, Switzerland, 2016; pp. 237–279.
36. Chauhan, H.; Bagyaraj, D.J.; Selvakumar, G.; Sundaram, S.P. Novel plant growth promoting rhizobacteria—Prospects and potential. *Appl. Soil Ecol.* 2015, 95, 38–53. [CrossRef]
37. Lema-Rumińska, J.; Kulus, D.; Tymoszuk, A.; Miler, N.; Woźniak, A.; Wenda-Piesik, A. Physiological, biochemical, and biometrical response of cultivated strawberry and wild strawberry in greenhouse gutter cultivation in the autumn-winter season in Poland—Preliminary Study. *Acta Sci. Pol. Hortorum Cultus* 2021, 15, 44–55.
38. Ahmad, F.; Ahmad, I.; Khan, M.S. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.* 2008, 163, 173–181. [CrossRef]
39. Govindasamy, V.; Senthilkumar, M.; Magheshwaran, V.; Kumar, U.; Bose, P.; Sharma, V.; Annapurna, K. *Bacillus* and *Paenibacillus* spp.: Potential PGPR for sustainable agriculture. In *Plant Growth and Health Promoting Bacteria: Maheshwari, D.K., Ed.; Microbiology Monographs*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 333–364.
40. Ferreira, C.M.H.; Soares, H.M.V.M.; Soares, E.V. Promising bacterial genera for agricultural practices: An insight on plant growth-promoting properties and microbial safety aspects. *Sci. Total Environ.* 2019, 682, 779–799. [CrossRef]
41. Cueva-Yesquén, L.G.; Goulart, M.C.; Attili de Angelis, D.; Nopper Alves, M.; Fantinatti-Garboggini, F. Multiple plant growth-promotion traits in endophytic bacteria retrieved in the vegetative stage from passionflower. *Front. Plant Sci.* 2021, 11, 2282. [CrossRef]
42. Comeau, D.; Balthazar, C.; Novinsack, A.; Bouhamdani, N.; Joly, D.L.; Filion, M. Interactions between *Bacillus* spp., *Pseudomonas* spp. and *Cannabis sativa* promote plant growth. *Front. Microbiol.* 2021, 12, 2688. [CrossRef]
43. Andreolli, M.; Lampis, S.; Zapparoli, G.; Angelini, E.; Vallini, G. Diversity of bacterial endophytes in 3 and 15 year-old grapevines of *Vitis vinifera* cv. Corvina and their potential for plant growth promotion and phytopathogen control. *Microbiol. Res.* 2016, 183, 42–52. [CrossRef]
47. Castagno, L.N.; Estrella, M.J.; Sannazzaro, A.I.; Grassano, A.E.; Ruiz, O.A. Phosphate-solubilization mechanism and in vitro plant growth promotion activity mediated by *Pantoea eucalypti* isolated from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina). *J. Appl. Microbiol.* 2011, 110, 1151–1165. [CrossRef]

48. Spaepen, S.; Vanderleyden, J. Auxin and plant-microbe interactions. *Cold Spring Harb. Perspect. Biol.* 2011, 3, a001438. [CrossRef]

49. Rajkumar, M.; Ae, N.; Prasad, M.N.V.; Freitas, H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol.* 2010, 28, 142–149. [CrossRef]

50. Saeid, A.; Prochownik, E.; Dobrowolska-Iwanek, J. Phosphorus solubilization by *Bacillus* species. *Molecules* 2018, 23, 2897. [CrossRef]

51. Zahir, Z.A.; Munir, A.; Asghar, H.N.; Shaharoona, B.; Arshad, M. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J. Microbiol. Biotechnol.* 2008, 18, 958–963.

52. Aasfar, A.; Bargaz, A.; Yaakoubi, K.; Hilali, A.; Bennis, I.; Zerouali, Y.; Meftah Kadmiri, I. Nitrogen fixing *Azotobacter* species as potential soil biological enhancers for crop nutrition and yield stability. *Front. Microbiol.* 2021, 12, 354. [CrossRef]

53. Chandran, H.; Meena, M.; Swapnil, P. Plant growth-promoting rhizobacteria as a green alternative for sustainable agriculture. *Sustainability* 2021, 13, 10986. [CrossRef]

54. Kudoyarova, G.; Arkhipova, T.; Korshunova, T.; Bakaeva, M.; Loginov, O.; Dodd, I.C. Phytohormone mediation of interactions between plants and non-symbiotic growing bacteria promoting bacteria under edaphic stresses. *Front. Plant Sci.* 2019, 10, 1368. [CrossRef] [PubMed]

55. Angelini, G.; Ragni, P.; Esposito, D.; Giardi, P.; Pompili, M.L.; Moscardelli, R.; Giardi, M.T. A device to study the effect of space performance of *Pantoea agglomerans* isolated from *Duvalia velutina* species. *Cold Spring Harb. Perspect. Biol.*, 630–635. [CrossRef]

56. Dimkpa, C.; Weinand, T.; Asch, F. Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ.* 2009, 32, 1682–1694. [CrossRef]

57. Yang, J.; Jang, J.; Kleoppper, J.W.; Ryu, C.M. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* 2009, 14, 1–4. [CrossRef] [PubMed]

58. Timmusk, S.; Abd El-Daim, I.A.; Copolovici, L.; Tanilas, T.; Kännaste, A.; Behers, L.; Nevo, E.; Seisenbaeva, G.; Stenström, E.; Niinemets, Ü. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS ONE* 2014, 9, e96086. [CrossRef]

59. Wang, C.; Guo, Y.; Wang, C.; Liu, H.; Niu, D.; Wang, Y.; Guo, J. Enhancement of tomato (*Lycopersicon esculentum*) tolerance to drought stress by plant-growth-promoting rhizobacterium (*PGPR Bacillus cereus*) AR156. *J. Agr. Biotechnol.* 2012, 20, 1097–1105.

60. Shrinibanyan, S.; Khosravi, H.; Malakouti, M.J. Alleviation of drought stress in maize (*Zea mays*) by inoculation with *Azotobacter* strains isolated from semi-arid regions. *Appl. Soil Ecol.* 2019, 133, 138–145. [CrossRef]

61. Griffiths, R.I.; Whiteley, A.S.; O’Donnell, A.G.; Rodríguez, A.; Lejarraga, I. Physiological and community responses of established grassland bacterial populations to water stress. *Appl. Environ. Microbiol.* 2003, 69, 6961. [CrossRef]

62. Omar, S.A.; Eldenairy, M.E.; Abdelfattah, M.H.; Abd-Elhalim, H.M.; Wróbel, J.; Kalaji, H.M. Alteration in expression level of some growth and stress-related genes after rhizobacteria inoculation to alleviate drought tolerance in sensitive *Pisum sativum* species. *Environ. Exp. Bot.* 2011, 73, a001438. [CrossRef]

63. Kour, D.; Khan, S.S.; Kaur, T.; Kour, H.; Singh, G.; Yadav, A.; Yadav, A.N. Drought adaptive microbes as bioinoculants for the horticultural crops. *Heliyon* 2022, 8, e09493. [CrossRef] [PubMed]

64. Wang, C.; Guo, Y.; Wang, C.; Liu, H.; Niu, D.; Wang, Y.; Guo, J. Enhancement of wheat (*Triticum aestivum*) tolerance to drought stress by plant-growth-promoting rhizobacterium (*PGPR Pantoea agglomerans*) strain and its effect on rhizosphere soil aggregation. *Appl. Environ. Microbiol.* 1998, 64, 3740. [CrossRef]

65. Yang, J.; Jang, J.; Kleoppper, J.W.; Ryu, C.M. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* 2009, 14, 1–4. [CrossRef] [PubMed]

66. Timmusk, S.; Abd El-Daim, I.A.; Copolovici, L.; Tanilas, T.; Kännaste, A.; Behers, L.; Nevo, E.; Seisenbaeva, G.; Stenström, E.; Niinemets, Ü. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS ONE* 2014, 9, e96086. [CrossRef]

67. Wang, C.; Guo, Y.; Wang, C.; Liu, H.; Niu, D.; Wang, Y.; Guo, J. Enhancement of tomato (*Lycopersicon esculentum*) tolerance to drought stress by plant-growth-promoting rhizobacterium (*PGPR Bacillus cereus*) AR156. *J. Agr. Biotechnol.* 2012, 20, 1097–1105.

68. Amellal, N.; Burtin, G.; Bartoli, F.; Heulin, T. Colonization of wheat roots by an exopolysaccharide-producing *Pantoea agglomerans* strain and its effect on rhizosphere soil aggregation. *Appl. Environ. Microbiol.* 1998, 64, 3740. [CrossRef]

69. Ilyas, N.; Bano, A.; Iqbal, S.; Raja, N.I. Physiological, biochemical and molecular characterization of *Azospirillum lipoferum* sp. isolated from maize under water stress. *Pak. J. Bot.* 2012, 44, 71–80.

70. Masrah, Y.S.; Al-Turki, T.A.; Sayed, O.H. Photosynthetic adaptation, and survival strategy of *Duvalia velutina* sp. isolated from semi-arid environment. *Photosynthetica* 2015, 53, 555–561. [CrossRef]

71. Yan, N.; Zhang, Y.L.; Xue, H.M.; Zhang, X.H.; Wang, Z.D.; Shi, L.Y.; Guo, D.P. Changes in plant growth and photosynthetic performance of *Zizania latifolia* exposed to different phosphorus concentrations under hydroponic condition. *Photosynthetica* 2015, 53, 630–635. [CrossRef]

72. Wang, C.; Guo, Y.; Wang, C.; Liu, H.; Niu, D.; Wang, Y.; Guo, J. Enhancement of tomato (*Lycopersicon esculentum*) tolerance to drought stress by plant-growth-promoting rhizobacterium (*PGPR Bacillus cereus*) AR156. *J. Agr. Biotechnol.* 2012, 20, 1097–1105.

73. Li, Y.; Wang, C.; Wang, C.; Liu, H.; Niu, D.; Wang, Y.; Guo, J. Enhancement of tomato (*Lycopersicon esculentum*) tolerance to drought stress by plant-growth-promoting rhizobacterium (*PGPR Bacillus cereus*) AR156. *J. Agr. Biotechnol.* 2012, 20, 1097–1105.

74. Amellal, N.; Burtin, G.; Bartoli, F.; Heulin, T. Colonization of wheat roots by an exopolysaccharide-producing *Pantoea agglomerans* strain and its effect on rhizosphere soil aggregation. *Appl. Environ. Microbiol.* 1998, 64, 3740. [CrossRef]

75. Ilyas, N.; Bano, A.; Iqbal, S.; Raja, N.I. Physiological, biochemical and molecular characterization of *Azospirillum lipoferum* sp. isolated from maize under water stress. *Pak. J. Bot.* 2012, 44, 71–80.

76. Masrah, Y.S.; Al-Turki, T.A.; Sayed, O.H. Photosynthetic adaptation, and survival strategy of *Duvalia velutina* sp. isolated from semi-arid environment. *Photosynthetica* 2015, 53, 555–561. [CrossRef]

77. Yan, N.; Zhang, Y.L.; Xue, H.M.; Zhang, X.H.; Wang, Z.D.; Shi, L.Y.; Guo, D.P. Changes in plant growth and photosynthetic performance of *Zizania latifolia* exposed to different phosphorus concentrations under hydroponic condition. *Photosynthetica* 2015, 53, 630–635. [CrossRef]

78. Ware, M.A.; Tribulato, A.; Trillo, A.; Di Lorenzo, V.; Romano, D. Effects of water stress on gas exchange, water relations and leaf structure in two ornamental shrubs in the Mediterranean area. *Agronomy* 2019, 9, 381. [CrossRef]
74. Tari, I.; Camen, D.; Coradini, G.; Csiszár, J.; Fedusc, E.; Gémes, K.; Lazar, A.; Madosa, E.; Mihacea, S.; Poór, P.; et al. Changes in chlorophyll fluorescence parameters and oxidative stress responses of bush bean genotypes for selecting contrasting acclimation strategies under water stress. *Acta Biol. Hung.* 2008, 59, 335–345. [CrossRef]

75. Ohashi, Y.; Nakayama, N.; Saneoka, H.; Fujita, K. Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. *Biol. Plant.* 2006, 50, 138–141. [CrossRef]

76. Czyczyło-Mysza, I.; Myśkowski, B. Analysis of the impact of drought on selected morphological, biochemical, and physiological traits of rye inbred lines. *Acta Physiol. Plant.* 2017, 39, 87–94. [CrossRef]

77. Razavi, F.; Pollet, B.; Steppe, K.; Van Labeke, M.C. Chlorophyll fluorescence as a tool for evaluation of drought stress in strawberry. *Photosynthetica* 2008, 46, 631–633. [CrossRef]

78. Strasser, R.J.; Tsimilli-Michael, M.; Srivastava, A. Analysis of the chlorophyll a fluorescence transient. In *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Advances in Photosynthesis and Respiration; Papageorgiou, G.C., Govindjee, Eds.; Springer: Dordrecht, Holand, 2004; pp. 321–362.

79. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A. Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.* 2009, 29, 185–212. [CrossRef]

80. Porcel, R.; Zamarreño, Á.; García-Mina, J.; Aroca, R. Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. *BMC Plant Biol.* 2014, 14, 36. [CrossRef] [PubMed]

81. Barnawal, D.; Bharti, N.; Pandey, S.S.; Pandey, A.; Chanotiya, C.S.; Kalra, A. Plant growth promoting rhizobacteria enhances wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. *Physiol. Plant.* 2017, 161, 502–514. [CrossRef]

82. Khan, N.; Bano, A.; Rahman, M.A.; Guo, J.; Kang, Z.; Babar, M.A. Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in chickpea (*Cicer arietinum* L.) induced by PGPR and PGRs. *Sci. Rep.* 2019, 9, 2097. [CrossRef]

83. Zarei, T.; Moradi, A.; Kazemeini, S.A.; Akhgar, A.; Rahi, A.A. The role of ACC deaminase producing bacteria in improving sweet corn (*Zea mays* L. var. *saccharata*) productivity under limited availability of irrigation water. *Sci. Rep.* 2020, 10, 20361. [CrossRef]

84. Pikovskaya, R.I. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Mikrobiologya* 1948, 17, 362–370.

85. Schwyn, B.; Neilands, J.B. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* 1987, 160, 47–56. [CrossRef]

86. Alexander, D.B.; Zuberer, D.A. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol. Fertil. Soils* 1991, 12, 39–45. [CrossRef]

87. Khalid, A.; Arshad, M.; Zahir, Z.A. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.* 2004, 96, 473–480. [CrossRef] [PubMed]

88. Dworkin, M.; Foster, J. Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol.* 1958, 75, 592–603. [CrossRef] [PubMed]

89. 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]

90. Honna, M.; Shimomura, T. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric. Biol. Chem.* 1978, 42, 1825–1831. [CrossRef]

91. Kalaji, M.H.; Bosa, K.; Kościelniak, J.; Żuk-Gołaszewska, K. Effects of salt stress on photosystem II 554 efficiency and CO₂ assimilation of two Syrian barley landraces. *Environ. Exp. Bot.* 2011, 73, 64–72. [CrossRef]

92. Dorman, C. *Environmental Data Analysis*; Springer International Publishing: Cham, Switzerland, 2020.

93. Jamiołkowska, A.; Skwaryło-Bednarz, B.; Kisielewski, A.; Walczak, A.; Bańka, B. Effect of AAC deaminase-containing plant growth-promoting rhizobacteria on the growth and yield of wheat. *Environ. Exp. Bot.* 2011, 73, 64–72. [CrossRef]

94. De Sá, J.P.M. *Applied Statistics Using SPSS, Statistica, MatLab and R*, 2nd ed.; Springer Science & Business Media: Berlin, Germany, 2007.