



Article Effect of Plant Growth-Promoting Bacteria Azospirillum brasilense on the Physiology of Radish (Raphanus sativus L.) under Waterlogging Stress

Gisselle Salazar-Garcia¹, Helber Enrique Balaguera-Lopez² and Juan Pablo Hernandez^{1,3,*}

- ¹ Biology Program, Environmental Microbiology Laboratory, Universidad El Bosque, Av. Cra. 9 No. 131 A-02, Bogota 110121, Colombia; gissellesalazarg8@gmail.com
- ² Departamento de Agronomía, Facultad de Ciencias Agrarias, Sede Bogotá, Universidad Nacional de Colombia, Carrera 30 No 45-03 Edificio 500, Bogota 111321, Colombia; hebalagueral@unal.edu.co
- ³ The Bashan Institute of Science, 1730 Post Oak Court, Auburn, AL 36830, USA
- * Correspondence: jphernandezs@unbosque.edu.co

Abstract: Stress due to waterlogging is considered an abiotic factor that negatively affects crop production, which, together with the excessive fertilization of crops, reduces cost-effectiveness and generates the need to create sustainable alternatives economically and environmentally. The effect of inoculation with *Azospirillum brasilense* on the physiology of the *Raphanus sativus* var. Crimson Giant subjected to waterlogging, was evaluated. Stomatal conductance, chlorophyll concentration and chlorophyll *a* fluorescence were analyzed to establish this effect, corroborating the beneficial effect of inoculation with *A. brasilense* in radish under waterlogging stress. The stomatal conductance of inoculated and waterlogged treatments presented the same values as the control plants, and photosystem II efficiency was favored in inoculated and waterlogged treatments (0.6 Fv/Fm) compared to non-inoculated and waterlogged treatments (0.3 Fv/Fm). The results suggested that this increased efficiency was due to the preservation of photosynthetic pigments in the tissues, allowing the preservation of stomatal conductance and a reduction in the amount of energy dissipated in the form of heat (fluorescence) due to inoculation with *A. brasilense*. Therefore, plant growth-promoting bacteria are responsible for activating and improving some physiological mechanisms of the plant.

Keywords: *Azospirillum;* PGPB; *Raphanus sativus;* stress physiology; chlorophyll a fluorescence; waterlogging stress

1. Introduction

Radish (*Raphanus sativus* L.) is a plant of the Brassicaceae family, with important nutritional properties due to its high vitamin and mineral contents [1]. This plant has phytochemicals, such as glucosinolate, myrosinase and isothiocyanate, alkaloids and nitrogen compounds, coumarins (hydroxycoumarin aesculetin and scopoletin), organic acids, phenolic compounds and sulfur compounds, among others. Radish has biological activities, including the production of antimicrobial, antiviral, antitumor, antioxidant [2,3], antihyperlipidemic [4], and antihyperglycemic agents with the possibility of reducing diabetes and associated consequences [5]. Furthermore, radish is a fast-growing plant that can be harvested 25 to 35 days after sowing, a situation that makes it very attractive for small and medium vegetable producers [6,7]. The high precocity of this species is also an important aspect from an experimental point of view, as it allows researchers to obtain results of its physiological behavior in a short time.

Soil waterlogging has long been identified as major abiotic stress, and the constraints it imposes on roots have a marked effect on plant growth and development due to a decrease in oxygen in the soil by floods and an excess of rainfall [8–10]. Plants are known to wilt more rapidly in waterlogged soil due to decreased hydraulic conductance in the



Citation: Salazar-Garcia, G.; Balaguera-Lopez, H.E.; Hernandez, J.P. Effect of Plant Growth-Promoting Bacteria *Azospirillum brasilense* on the Physiology of Radish (*Raphanus sativus* L.) under Waterlogging Stress. *Agronomy* 2022, *12*, 726. https:// doi.org/10.3390/agronomy12030726

Academic Editor: José David Flores-Félix

Received: 21 January 2022 Accepted: 21 February 2022 Published: 17 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). roots [11]. Waterlogging induces several physiological damages, including a reduction in rate photosynthesis, stomatal closure and growth inhibition of leaves, stem and roots, resulting in low yield and quality in numerous plant communities [12]. To minimize the effects of stress due to different abiotic conditions, many studies presented the possibility of using bacteria that can improve the ability of plants to grow in different conditions, such as waterlogging. Plant growth-promoting bacteria (PGPB; [13]) are defined as free-living soil, rhizosphere, rhizoplane, and phyllosphere bacteria that, under some conditions, are beneficial to plants [14]. Different studies show that, when plants are inoculated with PGPB, they increase their growth and many other physiological aspects, and even have the ability to tolerate stress conditions better. For example, when Zea mays with a nitrogen deficit was inoculated with Bacillus sp., an increase in the photosynthetic rate, leaf nitrate content and photosystem II (PSII) efficiency was found [15]. In Ocimum sanctum, inoculation with PGPB (Achomobacter xylosoxidans, Serratia ureilytica, Herbaspirullum seropedicae and Ochromobactrum rhizosphaerae) produced aminocyclopropane-1-carboxylic acid deaminase. A. xylosoxidans reduced damage caused by waterlogging, induced detrimental changes like stress ethylene production, reduced chlorophyll concentration, higher lipid peroxidation, proline concentration and reduced foliar nutrient uptake [12].

Azospirillum brasilense is the most studied PGPB and appears to have a significant potential for commercial applications. Many reports have shown that *A. brasilense* may promote the growth and yield of numerous plant species, many of which are of agronomic or ecological importance [16–18].

In this study, radish was inoculated with the PGPB *Azospirillum brasilense* and subjected to waterlogging to determine the effect of inoculation on the different physiological processes of radish and to demonstrate that PGPB can modify the physiology of the plant so that it can withstand different environmental conditions.

2. Materials and Methods

The study was carried out at the facilities of the Biology Research Laboratory of Universidad El Bosque (INBIBO, Bogota, Colombia) with the radish seedlings var. Crimson Giant (*R. sativus* L.), with 14 days of germination and was inoculated at the time of transplantation with *A. brasilense* Cd DMS 1843 immobilized in alginate beads [19]. Radish var. Crimson Giant Crimson Giant has a harvest maturity of approximately 30 days and can reach a height of 10–13 cm. The root crop has a round upper part, is very firm and crisp in texture, with a red color and a diameter between 3.5 to 4 cm. Plants were transplanted into 14 oz. pots with Forza[®] Mix (Fercon, Cali, Colombia) substrate consisting mainly of Canadian peat and fertilized every 3 days until day 15 with a Nutriponic[®] (Walco S.A.S, Bogota, Colombia) solution designed to supply the essential elements. All treatments were maintained in a greenhouse at a temperature of 8 °C (night) to 18 °C (day), 75% relativity humidity, and 12/12 h photoperiod.

2.1. Experimental Design

A completely randomized experimental design with 4 treatments and 11 replications was used. Each experimental unit consisted of one plant for a total of 44 plants. Four treatments were established by randomization as follows (Table 1):

Treatment	Definitions	Symbols
T1	Non-inoculated, non-waterlogged (control)	NIN+NW
T2	Non-inoculated, waterlogged	NIN+W
T3	Inoculated, non-waterlogged	IN+NW
T4	Inoculated, waterlogged	IN+W

 Table 1. Definitions of the evaluated treatments.

Plants of treatments T2 and T4 were watered with a 3 cm layer of water above the substrate and kept for 21 days. After this time, waterlogged experimental units were drained, and the experiment was dismantled.

2.2. Inoculant and Inoculation Method

A pre-inoculum was prepared with *A. brasilense* Cd DMS 1843 in nutrient broth (Sigma-Aldrich, St. Louis, MO, USA). This strain was used in the experiments as it is known as the model bacterium for the study of PGPB's [20–22]. After 12 h at 30 ± 2 °C, the culture was washed three times with 0.85% saline solution and centrifuged at 4200 rpm (Spectrafuge 6C Compact; Labnet, Edison, NJ, USA). The optical density of the inoculum was adjusted to 1 with $\lambda_{540 \text{ nm}}$ to obtain a concentration of 1×10^9 colony-forming units/mL [23] (Bashan and Levanony 1985). Then, under aseptic conditions, 20 mL of this inoculum were mixed with 80 mL of 2% sodium alginate (0.2 g L⁻¹) until a homogeneous mixture was obtained [24]. This mixture was dripped on 2% CaCl₂ solution (0.2 g L⁻¹), generating 100 g of beads with the inoculum, which was refrigerated until the moment of inoculation. The plants were inoculated during transplantation, 5 g of beads with the inoculum were placed in the substrate under the plant, and each plant was finally covered with 3 cm more of the substrate. Waterlogging was performed 7 days after inoculation.

2.3. Physiological Measurements

Periodic data collection was carried out every 3 days, from days 9 to 21 after the start of the treatments. Stomatal conductance (gs) was measured on fully extended leaves from the middle of the plant, and readings were made between 9 to 11 a.m. using a leaf porometer (model SC-1; Decagon Devices, Pullman, WA, USA). In these same leaves, the chlorophyll index was measured using a Fieldscout CM1000 chlorofilometer (Spectrum, Collinsville, IL, USA), and fluorescence parameters were measured using a PAM fluorometer (Walz, Effeltrich, Germany) under controlled light exposure conditions and with a light pulse modulated to less than 50 μ mol m⁻² s⁻¹. Previously, leaves were kept in the dark for 20 min. Using Wincontrol-3 software (Walz), the ratio of the maximum efficiency of the PSII (Fv/Fm), electron transport rate (ETR), photochemical quenching (Pq), and non-photochemical quenching (NPq) were recorded.

2.4. Growth Measurements

At the end of the experiment (21 days after the start of waterlogging), the plants were harvested in 5 randomly selected experimental units of each treatment. The following parameters were measured: the equatorial diameter of the tuberous root (mm) was measured using a vernier caliper, the number of leaves was directly counted, the leaf area (cm^2) was measured using a CID Bio-Science meter (Cl-202; Camas, WA, USA), leaves were put on the equipment display and then the scan was performed through the equipment sensor, and the fresh and dry weight of the plants were measured using a 0.0001 g precision balance. To calculate the dry weight, the samples were packed in paper bags and dried in an oven (Pinzuar, Madrid, Colombia) at 80 °C until constant weight.

2.5. Statistical Analysis

To evaluate the effect of the treatments, the results were subjected to an analysis of variance (ANOVA) when the assumptions of normality (Shapiro–Wilks test) and variance homogeneity (Levene's test) were met. The statistical differences of the means were analyzed according to Tukey's test (p < 0.05) with SPSS version 19 (IBM SPSS Statistics, New York, NY, USA).

3. Results

3.1. Physiological Parameters

3.1.1. Stomatal Conductance

Stomatal conductance showed no significant differences in radish on days 9 and 12 after waterlogging for any treatment. From day 12 to the end of the experiment (21 days after waterlogging), there were statistical differences between treatments. The inoculated plants (with and without waterlogging) showed the highest stomatal conductance from day 15. NIN+W plants had the lowest values and showed a continuous decrease in this parameter (71.7 \pm 7.3 mmol H₂O m⁻² s⁻¹ on day 21) (Figure 1).



Figure 1. Stomatal conductance in *R. sativus* under waterlogged conditions and inoculated with *A. brasilense*. Averages followed by different letters at each sampling point show statistical differences according to Tukey's test (p < 0.05). Vertical bars in each average indicate standard error (n = 11).

3.1.2. Chlorophyll Index

There were statistical differences in most of the samples except on day 12, where all treatments showed the same chlorophyll index. NIN+W plants always showed the lowest chlorophyll values. On day 21, the chlorophyll index was 85.8 ± 26.8 . In contrast, the plants of the other treatments showed significantly higher values, which also increased from day 15 (Figure 2).





Significant differences were observed on all sampling days. Inoculated plants (with and without waterlogging) presented similar values to control plants throughout the study. In all cases, these plants presented higher values than the plants that were waterlogged but not inoculated, which had very low values from day 15 (Figure 3).



Figure 3. Maximum efficiency of PSII in *R. sativus* under waterlogged conditions and inoculated with *A. brasilense*. Averages followed by different letters at each sampling point show statistical differences according to Tukey's test (p < 0.05). Vertical bars in each average indicate standard error (n = 11).

3.1.4. Photochemical Quenching (Pq)

Pq showed significant differences after 15 days of waterlogging. In waterlogged plants (even those inoculated), this parameter decreased significantly relative to non-waterlogged plants, with even lower values in NIN+W plants (Figure 4).



Figure 4. Pq in *R. sativus* under waterlogged conditions and inoculated with *A. brasilense*. Averages followed by different letters at each sampling point show statistical differences according to Tukey's test (p < 0.05). Vertical bars in each average indicate standard error (n = 11).

3.1.5. Non-Photochemical Quenching (NPq)

Differences were evident only on days 9 and 12 after waterlogging. In these samples, plants under waterlogging stress showed the highest values on days 9 and 12, especially those that were not inoculated. After day 12, NPq decreased and showed statistically similar values to the other treatments (Figure 5).



Figure 5. NPq in *R. sativus* under waterlogged conditions and inoculated with *A. brasilense*. Averages followed by different letters at each sampling point show statistical differences according to Tukey's test (p < 0.05). Vertical bars in each average indicate standard error (n = 11).

3.1.6. Electron Transport Rate (ETR)

It was evident that the highest response of this parameter was found in non-waterlogged plants with and without inoculation, but the effect was significant only after 12 days. On day 15, treatment with waterlogging and inoculation presented an ETR similar to non-waterlogging treatments (Figure 6).



Figure 6. ETR in *R. sativus* under waterlogged conditions and inoculated with *A. brasilense*. Averages followed by different letters at each sampling point show statistical differences according to Tukey's test (p < 0.05). Vertical bars in each average indicate standard error (n = 11).

3.2. Growth Parameters

Inoculated plants (with and without waterlogging) and plants without waterlogging showed the highest root diameter, number of leaves, and leaf area. The opposite response was evident in NIN+NW plants (Table 2). In fresh and dry weight measurements, non-waterlogged plants (with and without inoculation) showed the highest weight, whereas NIN+W plants produced the lowest weight. It is important to mention that IN+W plants showed an increased weight (Table 2).

Treatment	Diameter of Tuberous Root (mm)	Number of Leaves	Leaf Area (cm ²)	Fresh Weight (g)	Dry Weight (g)
NIN+NW	$31.86\pm4.15~^{\rm a}$	8.11 ± 0.56 $^{\rm a}$	277.34 ± 37.06 ^a	$29.87\pm2.07~^{ab}$	1.90 ± 0.17 $^{\rm a}$
NIN+W	12.08 ± 3.53 ^b	4.67 ± 1.03 ^b	86.03 ± 26.67 ^b	$3.42\pm1.30\ ^{\mathrm{c}}$	$0.16\pm0.06~^{ m c}$
IN+NW	37.53 ± 1.45 a	9.33 ± 0.37 a	274.22 ± 11.94 a	34.62 ± 1.65 a	1.83 ± 0.09 a
IN+W	33.04 ± 1.64 a	8.89 ± 0.26 $^{\rm a}$	$232.43\pm14.69~^{a}$	$23.68\pm2.78~^{b}$	1.25 ± 0.17 $^{\rm b}$
Significance	*	*	*	*	*

Table 2. Effect of inoculation with *A. brasilense* on the growth parameters of radish under waterlogging stress.

ANOVA: * significantly different at the 0.05 probability levels. Means with different letters represent statistically significant differences in each variable according to Tukey's test ($p \le 0.05$). \pm standard error (n = 5).

4. Discussion

4.1. Stomatal Conductance

Waterlogged radish decreased stomatal conductance. It is known that the closure of stomata occurs in response to the stressful factor of waterlogging; thus, the plant manages to reduce the loss of water by transpiration, generating the reduction in stomatal conductance [25]. In *Vigna radiata*, a decrease of up to 62% occurred during the watering period [26].

Radish inoculated with *A. brasilense* presented greater stomatal conductance. This means that bacteria allowed flooded plants to generate mechanisms to cope with the stressful period, which gave them an advantage compared to those not inoculated (1). This advantage may also explain why inoculation mitigated the effect of waterlogging on growth (Table 2). Cohen et al. [27] experimentally demonstrated that *Azospirillum* possesses the necessary biochemical machinery to produce abscisic acid, thus providing the opportunity to overcome the stress period [28]. However, this would not explain why radish showed greater stomatal conductance when IN+W, and it becomes a subject for further investigation.

In a similar study conducted on rice, inoculation with *Azospirillum* did not produce differences in stomatal conductance of the plant by itself. Still, when inoculation was combined with irrigation of the plants, the physiological variables of rice increased up to 35% compared to those that were not inoculated [29].

4.2. Chlorophyll Index

The negative effect of waterlogging on this parameter was also reported in *Physalis peruviana* L., where the chlorophyll concentration decreased by about 30% [30]. The physiological imbalance generated by waterlogging reduced the synthesis of chlorophyll and accelerated its degradation. In melon, the decrease in photosynthetic rate, as a consequence of the waterlogging period, resulted in the degradation of chlorophylls [31]. In corn, a decrease of up to 70% in the percentage of chlorophyll was recorded after the start of waterlogging [32].

In contrast, Kumar et al. [33] argued that inoculation with *A. brasilense* significantly increased the photosynthetic pigments of the plants; after the physiological response to stress conditions, the additive effects of the inoculation allowed for an increase in phytohormones involved in the production of these pigments. Similar results were reported by Davaran-Hagh et al. [34], who found an 8% increase in the content of chlorophyll in corn after inoculation.

It was found that stomatal conductance and chlorophyll index in waterlogged plants decrease [35], confirming that the opening of stomata can be a limiting factor of photosynthesis when the plants are subjected to stressful factors, such as waterlogging [36], and explains the relationship between leaf conductance and the chlorophyll index. Accordingly, the idea that inoculation with *A. brasilense* allows radish to cope with the waterlogging condition by keeping the stomata open and thus avoiding photodamage conditions and retaining the chlorophyll concentration in their tissues can be corroborated, even with similar values to the control plants (Figure 2). It is also possible to think that the result is due to an increase in aminocyclopropane-1-carboxylic acid deaminase produced by *A. brasilense*

that would reduce the production of ethylene and the damage caused by waterlogging, as reported in previous studies for PGPB [12].

4.3. Maximum Efficiency of PSII

NIN+W radish showed very low values (even <0.6, Figure 3), which indicated the serious damage produced by this stress on the photo phase of the photosynthesis process [37]. As in normal conditions, the maximum efficiency of PSII ranged from 0.7 to 0.8 [38]. Nauman et al. [39] argued that the maximum efficiency of PSII was drastically decreased after several days of waterlogging when damage to the leaves of the plants was already evident. This type of stress would cause damage to PSII of the flooded radish, possibly due to the stomatal closure presented, which was evidenced by a lower stomatal conductance. This, in turn, was due to the decoupling with the photo phase that leads to the generation of reactive oxygen species (ROS) that damage the photosynthetic machinery and also because, in waterlogged plants, the levels of ethylene in the leaves are increased, which induces the degradation of chlorophylls and other biomolecules and even stimulates defoliation to avoid water loss.

In *Distylium chinense*, waterlogging produced a decrease in the maximum efficiency of PSII and was dependent on the waterlogging time [40]. Flórez-Velasco et al. [25] and Sánchez-Reinoso et al. [41] also reported a decrease in the maximum efficiency of PSII in *Solanum quitoense* plants subjected to waterlogging. In tomato, the values of the maximum efficiency of PSII were similar to those in the present study; as in radish, tomato that maintained normal levels of water in the soil had optimal levels for this variable compared to flooded plants [42].

Liu et al. [40] argued that a decrease in the maximum efficiency of PSII implied a photoinhibition effect of waterlogging on the tissues; however, when the values remained relatively constant, they indicated that the stressor did not affect the photochemical reactions of PSII. Accordingly, inoculation with *A. brasilense* represented a significant advantage to flooded radish, allowing it to cope with the stress-generating event and retain the maximum efficiency of PSII. In a study by Heidari and Golpayagani [43] on basil, the recorded maximum efficiency values for plants inoculated with PGPB organisms were always higher than those not inoculated.

4.4. Photochemical Quenching (Pq)

The photochemical dissipation value quantifies the amount of energy captured by the open PSII reaction centers and is used in the photochemical transport of electrons [34]. The average of this parameter in healthy plants is between 0.8 and 1 [44]. The values of waterlogged radish were below this range, even showing a reduction of about 50%, compared to the values of the plants not subjected to stress, indicating the serious damage caused by waterlogging on radish. In this regard, Liu et al. [40] recorded the gradual decrease in Pq as the waterlogging time in *D. chinense* increased until it reached 60%.

IN+W plants showed advantages of inoculation (Figure 4). In the beginning, the plants coped with stress for a longer time. After day 12, the levels decreased significantly. However, it allowed plants to retain an adequate number of open PSII reaction centers compared to flooded and non-inoculated plants, thus ensuring that the efficiency of the photochemical pathway is increased.

4.5. Non-Photochemical Quenching (NPq)

Waterlogging generates stomatal closure in radish; as a consequence, the photo phase is affected. To avoid photoinhibition, the plant activates different mechanisms, and one of them is to dissipate excess energy in the form of heat. This process is activated to avoid excessive light absorption and photoinhibition in plants with some degree of stress [45]. NPq values indicate that the excess radiant energy is being dissipated in the form of heat in PSII; this may be related to the formation of zeaxanthin in the tissues [46].

Higher NPq values were evidenced in flooded plants up to 12 days after watering. However, there was no mitigation effect by *A. brasilensis* (Figure 5), indicating that waterlogging initially affects this parameter, but it is not through this mechanism that *A. brasilensis* generates acclimatization to waterlogging in radish.

4.6. Electron Transport Rate (ETR)

This parameter was affected in waterlogged radish 12 days after stress onset (Figure 6), indicating the negative effect of this stress on the photo phase. In a study carried out on beans, the ETR decreased by 29% after the watering period. This reduction was associated with the damage to PSII, which resulted from ROS accumulation [47]. In *D. chinense*, the ETR was reduced by 60% after waterlogging. Additionally, the authors reported that the flow of electrons and the efficiency to transform energy were affected by waterlogging [40]. However, inoculation with *A. brasilense* made it possible to cope with the reduction in the ETR in waterlogged radish for up to 15 days, allowing a greater flow of electrons to the PSII to probably guarantee higher production rates of NADPH. This positive effect is related to the fact that *A. brasilensis* avoids damage to PSII (as mentioned above) and, in general, to the photosynthetic apparatus, possibly explained by the fact that it mitigates the effect of waterlogging and prevents stomatal closure. In general, the results demonstrate the improvement in physiological processes after inoculation when the plants are subjected to stressful conditions [48].

4.7. Plant Growth

4.7.1. Tuberous Root Diameter

Waterlogging negatively affected the diameter of the tuberous root (Table 2), indicating the harmful effect of this condition on the growth of this organ [10]. Various studies demonstrated the increase in the concentration of phytohormones after inoculation with *Azospirillum*, not only improving the physiology of the plant when conditions are favorable but also acting when the environment is unfavorable [2], as what possibly occurred with radish under waterlogged conditions. Inoculation with the genus *Azospirillum* and in general, with PGPB, implies an improvement in the yield of the plant due to the increased production of photosynthates [49], which are then distributed to the different parts of the plant to favor an increase in size, and in this case, of the tuberous root the organ that can be harvested in radish. The greater root diameter can also be attributed to the ability of the bacteria to generate phytohormones, such as indole-3-acetic acid and gibberellins, which are involved in root growth and development [50]. In this regard, Méndez-Gómez et al. [51] claimed that *Azospirillum* reduces the effects of stressors, such as waterlogging, thanks to its ability to synthesize phytohormones at extreme pH.

4.7.2. Number of Leaves

In similar studies of seedling waterlogging, the number of leaves is reduced in the same way as it occurs in radish seedlings. In *Populus angustifolia* seedlings, waterlogging generated a gradual decrease in the number of leaves according to the total time of exposure to the stressor factor [52]. In tomato, waterlogging produced plants with a lower number of leaves, and the number of dead leaves during the study was twice as high in flooded plants [53]. In previous studies, inoculation with *Azospirillum* generated similar results in the number of leaves of plants. In seedlings of *Theobroma cacao*, inoculation generated an average of five more leaves per plant [54]. In flooded plants, the number of leaves was directly affected (Table 2), but *A. brasilense* allowed inoculated radish to cope with the waterlogging condition. Flooded soils are known to decrease the redox potential and the amount of oxygen present, affecting the availability of nutrients for plants [55]. As a consequence, plants need to activate a series of physiological and morphological mechanisms that allow them to cope with the main stressor event, the prolonged replacement of the gas phase present in the soil with a liquid phase, which generates the loss of nutrients and minerals due to root washing [56]. However, the genus *Azospirillum* can fix atmospheric

nitrogen under microaerobic oxygen conditions. Méndez-Gómez et al. [51] argued that the contribution of nitrogen by *Azospirillum* was estimated at 10 kg N/ha/year, supporting the hypothesis that inoculation confers an advantage when it comes to increasing the resistance of plants, such as radish to stressful conditions.

4.7.3. Leaf Area

In waterlogged radish, a significant reduction was recorded in this parameter (Table 2), corroborating what was exposed by Moreno and Fischer [57], who argued that the leaf area is significantly reduced after waterlogging, as the roots reduce the transport of water and negatively induce cell turgor, affecting leaf development. Furthermore, waterlogging generates an energy crisis in the plant due to the limitation of a number of metabolic routes; therefore, the reduction in leaf area is a consequence of the energy savings that the plant makes to maintain the water balance.

However, inoculation with *A. brasilense* gave radish the ability to withstand the stressful condition and maintain leaf area due to the increased production of phytohormones [51]. It was found that *A. brasilense* increases the total area of the leaves, increasing the area for gas exchange; for example, in cocoa, *A. brasilense* promoted a significant increase in leaf area [54].

4.7.4. Fresh and Dry Weight

These growth parameters were negatively affected by waterlogging, but this negative effect was mitigated by the presence of *A. brasilense* (Table 2). The growth of waterlogged plants is directly influenced by the lack of oxygen in the rhizosphere; as a consequence of anoxia, there is a reduction in the biomass of plants due to a decrease in gas exchange in the leaves, which in turn decreases the rate of photosynthesis [47]. Reduced dry weight was also found in *P. peruviana* plants [58]. It is also known that the significant reduction in the weight of the plants can be attributed to the low availability of nitrogen in the soil once it is flooded, in addition to the physiological dysfunctionalities derived from stomatal closure and photosynthetic limitations [59].

However, inoculation with *A. brasilense* in radish showed a positive effect in waterlogged and non-waterlogged plants, indicating the important role of this bacterium in the growth of radish. Veresoglou and Menexes [60] argued that inoculation with *Azospirillum* increases the production of dry biomass in plants by up to 33%, as indole acetic acid synthesized by the bacteria is used by the plant. In similar studies, inoculation with *Azospirillum* increases the yield of the plants; in cocoa, the dry weight of inoculated plants increased by 44% compared to non-inoculated ones [54]. In corn, the fresh weight of inoculated plants was 30% greater than that of non-inoculated plants. This increase was also attributed to the ability of *A. brasilense* to fix atmospheric nitrogen [34].

In summary, this study demonstrates that physiological and growth parameters of radish under waterlogging stress are improved when inoculated with *A. brasilense*. These results contribute to the studies of bacterial inoculants for agricultural purposes in which stress biotic conditions are present, such as waterlogging.

Author Contributions: Conceptualization, H.E.B.-L. and J.P.H.; methodology, G.S.-G.; H.E.B.-L. and J.P.H.; validation, H.E.B.-L. and J.P.H.; formal analysis, G.S.-G.; investigation, G.S.-G.; H.E.B.-L. and J.P.H.; writing—original draft preparation, H.E.B.-L. and J.P.H.; writing—review and editing, J.P.H. 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: Due to the nature of this study, the data does not need to be confidential and can be shared.

Acknowledgments: We thank the Vice-Rectory of Research, Universidad El Bosque (Bogota, Colombia), for financing this research through the PCI-2015-8776 Project. We also thank Sergio A. Llano for his critical reading of the manuscript. This study is dedicated to the memory of researcher Yoav Bashan (1951–2018).

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- 1. Criollo, H.; García, J. Efecto de la densidad de siembra sobre el crecimiento de plantas de rábano (*Raphanus sativus* L.) bajo invernadero. *Rev. Colomb. Cienc. Hortíc.* **2009**, *3*, 210–222. [CrossRef]
- Kumar, A.B.V.; Saila, E.S.; Narang, P.; Aishwarya, M.; Raina, R.; Gautam, M.; Shankar, E.G. Biofunctionalization and biological synthesis of the ZnO nanoparticles: The effect of *Raphanus sativus* (white radish) root extract on antimicrobial activity against MDR strain for wound healing applications. *Inorg. Chem. Commun.* 2019, 100, 101–106. [CrossRef]
- Jani, D.K.; Goswami, S. Antidiabetic activity of Cassia angustifolia Vahl. and Raphanus sativus Linn. leaf extracts. J. Trad. Complement. Med. 2019, 10, 124–131. [CrossRef]
- 4. Jani, D.; Goswami, S.S. Ameliorative effect of *Raphanus sativus* and *Cassia angustifolia* in experimentally induced hyperlipidemia and cardiovascular risk reduction. *Int. J. PharmTech Res.* **2017**, *10*, 273–279. [CrossRef]
- 5. Banihani, S.A. Radish (*Raphanus sativus*) and diabetes. *Nutrients* **2017**, *9*, 1014. [CrossRef] [PubMed]
- 6. Filgueira, F.A.R. *Novo Manual de Olericultura: Agrotecnologia Moderna Na Produção e Comercialização de Hortaliças*, 3rd ed.; UFV: Viçosa, Brazil, 2008.
- Basilio, A.; de Sousa, L.; da Silva, T.; de Moura, J.; Gonçalves, A.; de Melo Filho, J.; Leal, Y.; Dias, T. Morfofisiología del rábano (*Raphanus sativus* L.) bajo estrés salino y tratamientos con ácido ascórbico. *Agron. Colomb.* 2018, 36, 257–265.
- Parent, C.; Capelli, N.; Berger, A.; Crèvecoeur, M.; Dat, J.F. An overview of plant responses to soil waterlogging. *Plant Stress* 2008, 2, 20–27.
- 9. Fischer, G.; Ramírez, F.; Casierra-Posada, F. Ecophysiological aspects of fruit crops in the era of climate change. A review. *Agron. Colomb.* **2016**, *34*, 190–199. [CrossRef]
- 10. Cardona, W.A.; Bautista, L.G.; Flórez-Velasco, N.; Fischer, G. Desarrollo de la biomasa y raíz en plantas de lulo (*Solanum quitoense* var. septentrionale) en respuesta al sombrío y anegamiento. *Rev. Colomb. Cienc. Hortíc.* **2016**, *10*, 53–65. [CrossRef]
- 11. Cassán, F.; Diaz-Zorita, M. *Azospirillum* sp. in current agriculture: From the laboratory to the field. *Soil Biol. Biochem.* **2016**, *103*, 117–130. [CrossRef]
- Barnawal, D.; Bharti, N.; Maji, D.; Chanotiya, C.S.; Kalra, A. 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene generation. *Plant Physiol. Biochem.* 2012, *58*, 227–235. [CrossRef]
- Olanrewaju, O.S.; Glick, B.R.; Babalola, O.O. Mechanisms of action of plant growth promoting bacteria. World J. Microbiol. Biotechnol. 2017, 33, 1–16. [CrossRef] [PubMed]
- 14. Glick, B.R. Introduction to plant growth-promoting bacteria. In *Beneficial Plant-Bacterial Interactions*; Springer: Cham, Switzerland, 2020; pp. 1–37.
- Calzavara, A.K.; Paiva, P.H.G.; Gabriel, L.C.; Oliveira, A.L.M.; Milani, K.; Oliveira, H.C.; Bianchini, E.; Pimenta, J.A.; de Oliveira, M.C.N.; Stolf-Moreira, R. Associative bacteria influence maize (*Zea mays* L.) growth, physiology and root anatomy under different nitrogen levels. *Plant Biol.* 2018, 20, 870–878. [CrossRef]
- Camilios-Neto, D.; Bonato, P.; Wassem, R.; Tadra-Sfeir, M.Z.; Brusamarello-Santos, L.C.; Valdameri, G.; Donatti, L.; Faoro, H.; Weiss, V.A.; Chubatsu, L.S.; et al. Dual RNA-seq transcriptional analysis of wheat roots colonized by Azospirillum brasilense reveals up-regulation of nutrient acquisition and cell cycle genes. *BMC Genom.* 2014, 15, 1–13. [CrossRef]
- Vidotti, M.S.; Lyra, D.H.; Morosini, J.S.; Granato, Í.S.C.; Quecine, M.C.; Azevedo, J.L.D.; Fritsche-Neto, R. Additive and heterozygous (dis) advantage GWAS models reveal candidate genes involved in the genotypic variation of maize hybrids to Azospirillum brasilense. *PLoS ONE* 2019, 14, e0222788. [CrossRef]
- 18. Peng, H.; de-Bashan, L.E.; Higgins, B.T. Comparison of algae growth and symbiotic mechanisms in the presence of plant growth promoting bacteria. *Algal Res.* **2021**, *53*, 102156. [CrossRef]
- 19. Bashan, Y.; de-Bashan, L.E. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment. In *Advances in Agronomy*; Academic Press: Cambridge, MA, USA, 2010; Volume 108, pp. 77–136.
- Bacilio, M.; Hernandez, J.P.; Bashan, Y. Restoration of giant cardon cacti in barren desert soil amended with common compost and inoculated with Azospirillum brasilense. *Biol. Fertil. Soils* 2006, 43, 112–119. [CrossRef]
- de-Bashan, L.E.; Hernandez, J.P.; Bashan, Y.; Maier, R.M. Bacillus pumilus ES4: Candidate plant growth-promoting bacterium to enhance establishment of plants in mine tailings. *Environ. Exp. Bot.* 2010, 69, 343–352. [CrossRef]
- Lopez, B.R.; Bashan, Y.; Trejo, A.; de-Bashan, L.E. Amendment of degraded desert soil with wastewater debris containing immobilized Chlorella sorokiniana and Azospirillum brasilense significantly modifies soil bacterial community structure, diversity, and richness. *Biol. Fertil. Soils* 2013, 49, 1053–1063. [CrossRef]

- 23. Bashan, Y.; Levanony, H. An improved selection technique and medium for the isolation and enumeration of *Azospirillum* brasilense. Can. J. Microbiol. **1985**, *31*, 947–952. [CrossRef]
- de-Bashan, L.E.; Hernandez, J.P.; Bashan, Y. Interaction of *Azospirillum* spp. with microalgae: A basic eukaryotic-prokaryotic model and its biotechnological applications. In *Handbook for Azospirillum*; Springer: Cham, Switzerland, 2015; pp. 367–388.
- 25. Flórez-Velasco, N.; Balaguera-López, H.E.; Restrepo-Díaz, H. Effects of foliar urea application on lulo (*Solanum quitoense* cv. septentrionale) plants grown under different waterlogging and nitrogen conditions. *Sci. Hortic.* **2015**, *186*, 154–162. [CrossRef]
- 26. Ahmed, S.; Nawata, E.; Sakuratani, T. Changes of endogenous ABA and ACC, and their correlations to photosynthesis and water relations in mungbean (*Vigna radiata* (L.) Wilczak cv. KPS1) during waterlogging. *Environ. Exp. Bot.* **2006**, *57*, 278–284. [CrossRef]
- 27. Cohen, A.C.; Bottini, R.; Piccoli, P.N. *Azosporillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in *Arabidopsis* plants. *Plant Growth Regul.* **2008**, *54*, 97–103. [CrossRef]
- 28. Cassán, F.; Vanderleyden, J.; Spaepen, S. Physiological and agronomical aspects of phytohormone production by model plantgrowth-promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum*. J. Plant Growth Regul. 2014, 33, 440–459. [CrossRef]
- Ruiz-Sánchez, M.; Armada, E.; Muñoz, Y.; García-de Salamone, I.; Aroca, R.; Ruíz-Lozano, J.; Azcón, R. Azospirillum and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. J. Plant Physiol. 2011, 168, 1031–1037. [CrossRef] [PubMed]
- Villareal, A. Evaluación fisiológica de plantas de uchuva (*Physalis peruviana* L.), en la respuesta al estrés por anegamiento e infección de *Fusarium oxysporum*. In *Tesis de Maestría Facultad de Ciencias Agrarias*; Universidad Nacional de Colombia: Bogotá, Colombia, 2014.
- Yetisir, H.; Caliscan, M.; Soylu, S.; Sakar, M. Some physiological and growth responses of watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) grafted onto Lagenaria siceraria to flooding. *Environ. Exp. Bot.* 2006, 58, 1–8. [CrossRef]
- 32. Ren, B.; Zhang, J.; Dong, S.; Liu, P.; Zhao, B. Effects of waterlogging on leaf mesophyll cell ultrastructure and photosynthetic characteristics of summer maize. *PLoS ONE* **2016**, *11*, 1–22. [CrossRef]
- 33. Kumar, P.; Gupta, A.; Sharma, L.; Bakade, R. Mechanisms of Azospirillum in plant growth promotion. Sch. J. Agri. Vet. Sci. 2017, 4, 338–343.
- 34. Davaran-Hagh, E.; Mirshekari, B.; Reza-Ardakani, M.; Farahvash, F.; Rejali, F. *Azospirillum lipoferum* and nitrogen fertilization effect on chlorophyll content, nutrients uptake and biometric properties of *Zea mays* L. *Agrociencia* 2015, 49, 889–897.
- 35. Castro-Duque, N.E.; Chávez-Arias, C.C.; Restrepo-Díaz, H. Foliar glycine betaine or hydrogen peroxide sprays ameliorate waterlogging stress in cape gooseberry. *Plants* **2020**, *9*, 644. [CrossRef]
- 36. Striker, G.G. Flooding stress on plants: Anatomical, morphological and physiological responses. *Botany* 2012, 1, 3–28.
- Jiménez-Suancha, S.C.; Alvarado, O.H.; Balaguera-López, H.E. Fluorescencia como indicador de estrés en *Helianthus annuus* L. Una revisión. *Rev. Colomb. Cienc. Hortíc.* 2015, *9*, 149–160. [CrossRef]
- 38. Maxwell, K.; Johnson, G. Chlorophyll fluorescence—A practical guide. J. Exp. Bot. 2000, 51, 659–668. [CrossRef]
- 39. Nauman, J.; Young, D.; Anderson, J. Leaf chlorophyll fluorescence, reflectance, and physiological response to freshwater and saltwater flooding in the evergreen shrub, *Myrica cerifera*. *Environ*. *Exp. Bot.* **2008**, *63*, 402–409. [CrossRef]
- 40. Liu, Z.; Cheng, R.; Xiao, W.; Guo, Q.; Wang, N. Effect of off-season flooding on growth, photosynthesis, carbohydrate partitioning, and nutrient uptake in *Distylium chinense*. *PLoS ONE* **2014**, *9*, 1–9. [CrossRef]
- Sánchez-Reinoso, A.D.; Jiménez-Pulido, Y.; Martínez-Pérez, J.P.; Pinilla, C.S.; Fischer, G. Parámetros de fluorescencia de la clorofila y otros parámetros fisiológicos como indicadores del estrés por anegamiento y sombrío en plántulas de lulo (*Solanum quitoense* var. septentrionale). *Rev. Colomb. Cienc. Hortíc.* 2019, 13, 325–335. [CrossRef]
- 42. Else, M.; Janowiak, F.; Atkinson, C.; Jackson, M. Root signals and stomatal closure in relation to photosynthesis, chlorophyll *a* fluorescence and adventitious rooting of flooded tomato plants. *Ann. Bot.* **2009**, *103*, 313–323. [CrossRef] [PubMed]
- 43. Heidari, M.; Golpayegani, A. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). J. Saudy Soc. Agric. Sci. 2012, 11, 57–61. [CrossRef]
- 44. Mohammed, G.H.; Zarco-Tejada, P.; Miller, J.R. Applications of chlorophyll fluorescence in forestry and ecophysiology. In *Practical Applications of Chlorophyll Fluorescence in Plant Biology*; Springer: Boston, MA, USA, 2003; pp. 79–124.
- Goltsev, V.; Kalaji, H.; Paunov, M.; Baba, W.; Horaczek, T.; Mojski, J.; Kociel, H.; Allakhverdiev, S. Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. *Russ. J. Plant Physiol.* 2016, 63, 869–893. [CrossRef]
- Dall'Osto, L.; Holt, N.; Kaligotla, S.; Fuciman, M.; Cazzaniga, S.; Carbonera, D.; Frank, H.A.; Alric, J.; Basi, R. Zeaxanthin protects plant photosynthesis by modulating chlorophyll triplet yield in specific light-harvesting antenna subunits. *J. Biol. Chem.* 2012, 287, 41820–41834. [CrossRef]
- Velasco, N.F.; Ligarreto, G.A.; Díaz, H.R.; Fonseca, L.P.M. Photosynthetic responses and tolerance to root-zone hypoxia stress of five bean cultivars (*Phaseolus vulgaris* L.). S. Afr. J. Bot. 2019, 123, 200–207. [CrossRef]
- 48. Azooz, M.; Ahmad, P. Plant Environment Interaction: Responses and Approaches to Mitigate Stress; Wiley-Blackwell: Hoboken, NJ, USA, 2015.
- 49. Bashan, Y.; Bustillos, J.J.; Leyva, L.A.; Hernandez, J.P.; Bacilio, M. Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by *Azospirillum brasilense*. *Biol. Fertil. Soils* **2006**, *42*, 279–285. [CrossRef]
- 50. D'Angioli, A.; Gorne, R.; Lambers, H.; Frankland, A.; Silva, R. Inoculation with *Azospirillum brasilense* (Ab-V4, Ab-V5) increases *Zea mays* root carboxylate-exudation rates, dependent on soil phosphorus supply. *Plant Soil* **2017**, *410*, 499–507. [CrossRef]
- 51. Méndez-Gómez, M.; Castro-Mercado, E.; García-Pineda, E. *Azospirillum* una rizobacteria con uso potencial en la agricultura. *Biologicas* 2014, 16, 11–18.

- 52. Rood, S.; Nielsen, J.; Shenton, L.; Gill, K.; Letts, M. Effects of flooding on leaf development, transpiration, and photosynthesis in narrow leaf cottonwood, a willow-like poplar. *Photosynth. Res.* **2010**, *104*, 31–39. [CrossRef] [PubMed]
- Baracaldo, A.; Carvajal, R.; Romero, A.; Prieto, A.; Garcia, F.; Fisher, G.; Miranda, D. El anegamiento afecta el crecimiento y producción de biomasa en tomate chonto (*Solanum lycopersicum* L.), cultivado bajo sombrío. *Rev. Colomb. Cienc. Hortíc.* 2014, 8, 92–102. [CrossRef]
- Aguirre-Medina, J.; Mendoza-López, A.; Cadena-Iniguez, J.; Avendaño-Arrazate, C. Efecto de la biofertilización en vivero del cacao (*Theobroma cacao* L) con *Azospirillum brasilense* tarrand, Krieg et Döbereiner y *Glomus intraradices* Schenk et Smith. *Interciencia* 2007, 32, 541–556.
- 55. De la Cruz-Jiménez, J.; Moreno, P.; Magnitskiy, S. Respuesta de las plantas a estrés por inundación. Una revisión. *Rev. Colomb. Cienc. Hortíc.* 2012, 6, 96–109. [CrossRef]
- 56. Pardos, J.A. Respuestas de las plantas al anegamiento del suelo. Invest Agrar. Sist. Recur. 2008, 13, 101–107. [CrossRef]
- 57. Moreno, A.; Fischer, G. Efectos del anegamiento en los frutales. Una Revisión. Temas Agrar. 2014, 19, 106–123. [CrossRef]
- 58. Aldana, F.; García, P.; Fischer, G. Effect of waterlogging stress on the growth, development and symptomatology of cape gooseberry (*Physalis peruviana* L.) plants. *Rev. Acad. Colomb. Cienc. Ex. Fis. Nat.* **2014**, *38*, 393–400. [CrossRef]
- 59. Schramm, M.; Schaffer, B. Photosynthetic and growth responses of *Eugenia uniflora* L. seedlings to soil flooding and light intensity. *Environ. Exp. Bot.* **2010**, *68*, 113–121.
- 60. Veresoglou, S.; Menexes, G. Impact of inoculation with *Azospirillum* spp. on growth properties and seed yield of wheat: A meta-analysis of studies in the ISI Web of Science from 1981 to 2008. *Plant Soil* **2010**, *337*, 469–480. [CrossRef]