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ORIGINAL RESEARCH ARTICLE

Growth and photochemical efficiency of photosystem ii in seedlings of 2 varieties of *Capsicum annuum* L. inoculated with rhizobacteria or arbuscular mycorrhizal fungi

Azareel Angulo-Castro[1], Ronald Ferrera-Cerrato[1*], Alejandro Alarcón[1], Juan José Almaraz-Suárez[1], Julián Delgadillo-Martínez[1], Maribel Jiménez-Fernández[2], Oscar García-Barradas[3]

*[*1] Microbiología, Edafología, Campus Montecillo, Colegio de Posgraduados, Texcoco, Estado de México, Mexico. E-mail: rferreracerrato@gmail.com*

[2] Instituto de Ciencias Básicas, Universidad Veracruzana, Xalapa, Veracruz, Mexico.

[3] Unidad de Servicios de Apoyo en Resolución Analítica (SARA), Universidad Veracruzana, Xalapa, Veracruz, Mexico.

ABSTRACT

An alternative for sustainable management in the cultivation of *Capsicum annuum* L. has focused on the use of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF). This research selected PGPRPGPR and AMF based on their effect on Bell Pepper and Jalapeño bell pepper plants. Five bacterial strains isolated from different localities in the state of Mexico (P61 [*Pseudomonas tolaasii*], A46 [*P. tolaasii*], R44 [*Bacillus pumilus*], BSP1.1 [*Paenibacillus* sp.] and OLs-Sf5 [*Pseudomonas* sp.]) and 3 AMF treatments (H1 [consortium isolated from Chile rhizosphere in the state of Puebla], H2 [*Rhizophagus intraradices*] and H3 [consortium isolated from lemon rhizosphere from the state of Tabasco]). In addition, a fertilized treatment (Steiner solution 25%) and an absolute control were included. Jalapeño bell pepper "Caloro" and Bell Pepper "California Wonder" seedlings were inoculated with AMF at sowing and with CPB 15 days after emergence, and grown under controlled environment chamber conditions. In Jalapeño bell pepper, the best bacterial strain was P61 and the best AMF treatment was H1; in Bell Pepper the best strain was R44 and the best AMF were H3 and H1. These microorganisms increased the growth of jalapeño bell pepper and Bell Pepper seedlings compared to the unfertilized control. Likewise, P61 and R44 positively benefited the photosynthetic capacity of PSII.

Keywords: Mycorrhizal Fungi; Rhizobacteria; Growth Promotion

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1. Introduction

Mexico is one of the main Chile bell pepper producing countries in the world and has the greatest genetic diversity of Capsicum[1,2]. The most cultivated Chile varieties in the north of the country are Bell Pepper and jalapeño, whose management demands high amounts of chemical fertilizers, which are not fully utilized by the plants. This results in high production costs and potential soil contamination[3–5]. Nitrogen fertilizers are the most widely used in horticultural crops and their overuse generates large-scale environmental impacts that endanger the sustainability of ecosystems by causing eutrophication and contributing to global warming, as they are an important source of nitrous oxide (N_2O)[6–8].

An alternative to the problem of overfertilization is the use of Plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), with which it is possible to develop environmentally friendly systems (ecological agriculture), reduce the application of

chemical fertilizers and maintain sustainable production[9]. PGPR cohabit in PGPR cohabit in the rhizosphere and can benefit plant health, stimulate plant growth and protect against pathogens[10]; in addition, they can reduce the effects of abiotic stress and favor crop yields by participating in nutrient recycling and soil fertility[11,12].

AMF are important in organic farming because of the benefits they have on crops by acting as mobilizers of water and nutrients, including phosphorus, zinc and copper, and as biological control agents[13–15]. They can also increase plant tolerance to various abiotic stress factors, such as drought, excessive levels of toxic elements, salinity, and nutrient imbalances or deficiencies[16–18]. Some vegetables that in their initiation require a nursery stage, as is the case of chili (*Capsicum annuum* L.), may have benefits from AMF inoculation[19].

Some studies show the benefit of PGPR and AMF on chili bell pepper seedlings. Flores *et al.*[20] observed that *Azospirillum brasilense* and *Pantoea dispersa* favored nitrogen nutrition and growth of bell pepper seedlings, especially when *Azospirillum* and *Pantoea* were combined with low levels of NO_3. Likewise, the bacteria *Klebsiella pneumoniae* and the AMF *Glomus intraradices* favored the height, root length and dry weight of chili bell pepper plants with respect to plants without inoculation[21]. Inoculation of *G. intraradices* and *Gigaspora margarita* on 8 different bell pepper genotypes under growth chamber conditions led to higher dry weight compared to non-inoculated plants[22]. In addition, the favorable effects of AMF colonization on the growth of *Capsicum annuum* cv. 11B 14 have been related to better adaptation to salinity conditions[23].

Despite the mentioned benefits, there is limited information on the effect of bacteria such as *Paenibacillus* sp., *Pseudomonas* sp. and *Bacillus pumilus*, as well as on the effect of arbuscular mycorrhizal consortia in the promotion of plant growth in chili bell pepper seedlings. Therefore, the present research focused on evaluating the effect of 5 strains of PGPR and some AMF on the growth and efficiency of PSII of Bell Pepper and jalapeño bell pepper seedlings, under controlled conditions.

2. Materials and methods

2.1 Plant material and experimental conditions

The experiment was carried out in a controlled environment chamber (28 °C, 70% relative humidity, 12 h photoperiod). Seeds of jalapeño bell pepper variety jalapeño M.P.A "Caloro" (Semillas Caloro, Mexicana Industrial de Insumos Agropecuarios S.A. de C.V., Guadalajara, Jalisco, Mexico) and seeds of Bell Pepper bell pepper variety "California Wonder" (Distribuidora Rancho Los Molinos S.A. DE C.V., Tepoztlan, Morelos, Mexico) were used, both with germination greater than 89%. Hereafter, these varieties will be referred to as Jalapeño Pepper and Bell Pepper, respectively.

2.2 Microbiological material

Bacterial strains A46 and P61 (*Pseudomonas tolaasii*), R44 (*B. pumilus*), BSP1.1 (*Paenibacillus* sp.) and OLs-Sf5 (*Pseudomonas* sp.) were used. In addition, 3 AMF inocula were used: H1, H2 and H3. H1 was a consortium isolated from Chile rhizosphere in the state of Puebla, composed of *Funneliformis aff. geosporum* and *Claroideoglomus* sp. After collection, this material, which contained 2,590 spores per 100 g of dry soil, was stored at 4 °C. Inoculum H2 consisted of fresh roots of trap culture with *Lolium multiflorum* grass colonized 86% by *Rhizofagus intraradices*. Inoculum H3 consisted of fresh roots of *L. multiflorum* with 93% colonization by an AMF consortium composed of *Rhizophagus fasciculatus*, *Glomus* sp. and *Archaeospora* sp. isolated from lemon rhizosphere from the state of Tabasco. These fungal consortia were considered in terms of the benefit they provide to their hosts; all of them are continuously propagated under greenhouse conditions for research use.

2.3 Preparation of bacterial inoculum

Each strain of bacteria grew in nutrient broth at 28 °C for 72 h. The obtained culture was centrifuged at 7,000 rpm for 15 min to separate the microbial concentrate, resuspended in sterile distilled water, and centrifuged twice again to remove the remaining nutrients. The concentration of bacterial cells in inoculum P61, R44, OLS-SF5, A46 and BSP1.1 was

1.68×10^8, 2.45×10^8, 3.73×10^8, 8.6×10^8 and 4.0×10^5 UFC/mL, respectively.

Some reported characteristics of PGPR genera or species used in this experiment are as follows: *P. Tolaasii* and *Pseudomonas* sp. produce auxin and dissolve phosphate[24,25]; *Paenibacillus* sp. and *B. Pumilus* promoted growth, dissolved phosphate and produced auxin[26,27]. These characteristics were confirmed in the strains used in this experiment. It was found that P61 and A46 dissolved phosphate and produced auxin and iron carrier, while R44, OLS-SF5 and BSP1.1 produced auxin and dissolved phosphate[28] (Almaraz Suarez, unpublished results).

2.4 Seeding and inoculation

Sowing was carried out in 200-cavity Styrofoam trays; these were cut into sections of 20 cavities each (one section per treatment). One seed was sown per cavity, using a substrate composed of sand, peat and perlite (2:1:1 v/v), previously sterilized (121 °C for 3 h, on 2 consecutive days).

Inoculation by AMF H2 and H3 was performed at the time of sowing by placing 0.5 g of grass (*L. multiflorum*) root fragments at a depth of 2 cm of the root ball. On the other hand, AMF H1 was mixed at the time of sowing with the substrate at a ratio of 1:4 v/v. Inoculation of the bacterial strains was carried out 15 days after germination of the plant material, placing 2 mL of inoculum per plant directed to the root; the control with fertilization (25% Steiner's solution) and the control without fertilization were maintained without inoculation. In the case of the control with fertilization, Steiner's solution adjusted to an electrical conductivity of 0.5 ds/m was used, with the following components (mg/L): $Ca(NO_3)_2$ $4H_2O$ (265.5); KNO_3 (78); K_2SO_4 (67.5); $MgSO_4$ $7H_2O$ (123) and KH_2PO_4 (34), with pH adjusted to 6.5.

2.5 Variables evaluated

After 70 days, plants were evaluated and harvested; plant height, stem diameter, root volume, number of leaves, leaf area, leaf and total dry weight, PSII photochemical efficiency (Fv/Fo) and mycorrhizal colonization were measured. Leaf area specific was estimated by dividing leaf area by leaf dry weight (cm/g). Root volume was measured using the water displacement technique[29]: the whole root was immersed in a graduated cylinder with a given volume of water, the volume of water displaced by the root was expressed in cm^3. Leaf area was determined with a LICOR leaf area meter (LI 3000, Inc. Lincoln, NE, USA). Dry biomass was obtained after drying (70 °C, 72 h) and weighing leaves, stems, and roots separately on an analytical balance (Sartorius, Model Analytic AC 210S, Illinois, USA). The photochemical efficiency of PSII (Fv/Fo) was measured with an fluorometer OS-30p + (Opti-Sciences), considering readings on the youngest fully developed leaf. The percentage of mycorrhizal colonization was evaluated with the thinning and staining technique[30].

2.6 Treatments and experimental design

The experiment contemplated a completely randomized experimental design with 10 treatments (5 PGPR strains, 3 AMF inoculums and 2 controls, one with chemical fertilization and the other as an absolute control, without fertilization) and 15 replicates each. This resulted in a total of 150 experimental units for each chili variety. The data were analyzed using the SAS program for Windows[31], performing an analysis of variance and a test for comparison of means (LSD, $\alpha = 0.05$).

3. Results

Figures 1 and **2** show some global growth parameters evaluated in Bell Pepper and jalapeño bell pepper, respectively. It is observed that treatments inoculated with AMF showed greater plant height in both chili cultivars (**Figures 1A** and **2A**); in Bell Pepper, the H1 treatment was superior ($p = 0.05$) to the other treatments (**Figure 1A**), while in jalapeño chili, H1 and H3 treatments led to greater plant height (**Figure 2A**). As for inoculation with bacteria, Bell Pepper plants inoculated with A46, P61, OLs-Sf5 and BSP1.1 showed greater height ($p = 0.05$) with respect to the control and the fertilized control (**Figure 1A**). In the case of jalapeño bell pepper, inoculation with bacteria did not produce significant effects on height compared to the control and the fertilized control (**Figure 2A**).

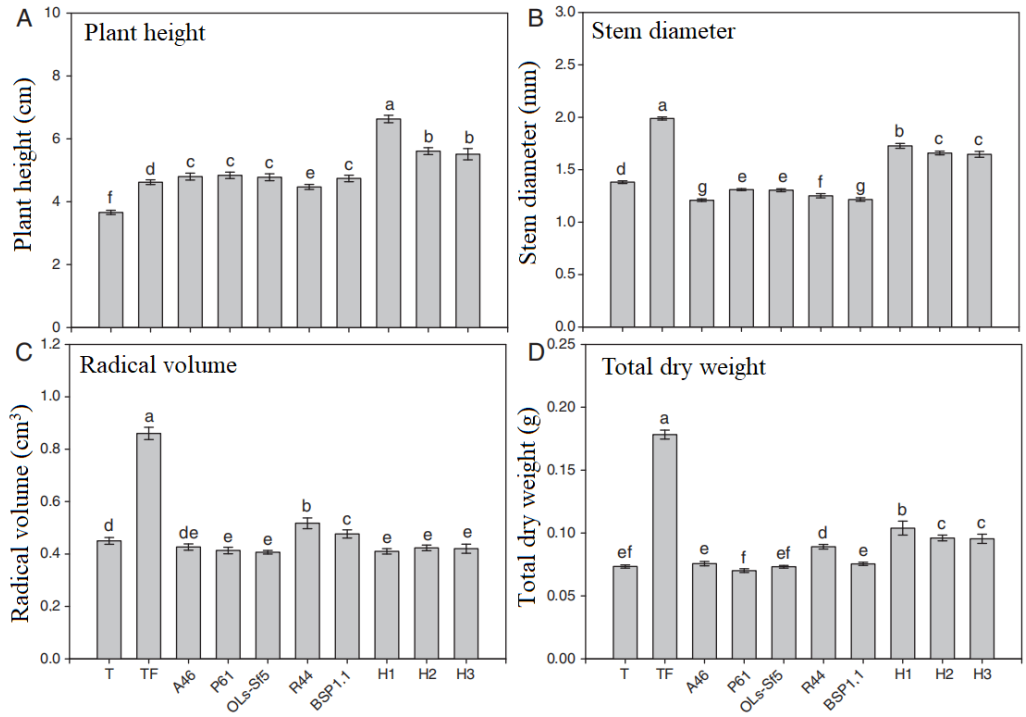

Figure 1. Height (**A**), stem diameter (**B**), root volume (**C**) and total dry weight (**D**) of Bell Pepper plants after 70 days. Means standard error are shown; n = 15. Identical letters above bars indicate no significant difference (LSD, α = 0.05). A46: *Pseudomonas tolaasii*; P61: Pseud*omonas tolaasii*; OLs-Sf5: Pseudomomas sp.; R44: Bacillus pumilus; BSP1.1: *Paenibacillus* sp.; H1: *Funneliformis aff. geosporum* and *Claroideoglomus* spp.; H2: *Rhizophagus intraradices*; H3: *Rhizophagus fasciculatus*, *Glomus* sp. and *Archaeospora* sp.; T: absolute control; TF: fertilized control.

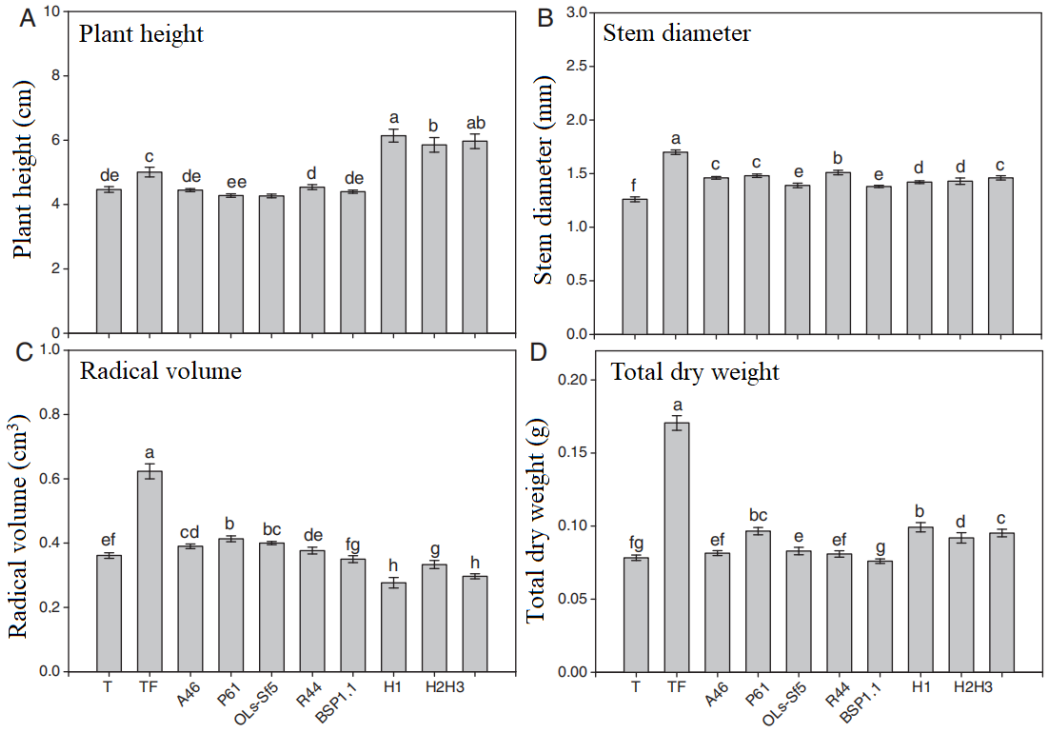

Figure 2. Height (**A**), stem diameter (**B**), root volume (**C**) and total dry weight (**D**) of jalapeño bell pepper plants after 70 days. Means are shown standard error; n = 15. Identical letters above the bars indicate no significant difference (LSD, α = 0.05). A46: *Pseudomonas tolaasii*; P61: *Pseudomonas tolaasii*; OLs-Sf5: *Pseudomomas* sp. R44: *Bacillus pumilus*; BSP1.1: *Paenibacillus* sp.; H1: *Funneliformis aff. geosporum* and *Claroideoglomus* spp.; H2: *Rhizophagus intraradices*; H3: *Rhizophagus fasciculatus*; *Glomus* sp. and *Archaeospora* sp.; T: absolute control; TF: fertilized control.

It can be noticed in the same figures that the fertilized controls were the treatments that showed greater stem diameter in both Chile bell pepper cultivars, as observed in **Figures 1B** and **2B**. In Bell Pepper, the treatment with H1 was superior ($p = 0.05$) to the other treatments, but inferior to the fertilized control (**Figure 1B**), while in Jalapeño bell pepper it was the inoculation with the H3 consortium that was superior in this parameter ($p = 0.05$) to the other AMF treatments (**Figure 2B**). On the other hand, inoculation of Bell Pepper with bacteria led in all cases to a smaller stem diameter ($p = 0.05$) compared to the control and the fertilized control (**Figure 1B**). The same was not true for jalapeño bell pepper, where inoculation with bacteria led to a higher stem diameter ($p = 0.05$) than in the unfertilized control; strain R44 showed the best performance in this respect compared to the rest of the bacterial inocula (**Figure 2B**).

Figures 1C and **2C** show that the fertilized control showed the greatest root volume in both chili bell pepper cultivars. Likewise, it is noticed in **Fgure 1C** that in Bell Pepper, the treatments inoculated with R44 and BSP1.1 bacteria showed greater root volume with respect to the inoculations A46, P61 and OLs-Sf5, also to the unfertilized control and to the treatments inoculated with AMF ($p = 0.05$). In jalapeño bell pepper plants, those inoculated with A46, P61 and OLs-Sf5 bacteria were the ones that exceeded the unfertilized control in root volume ($p = 0.05$) (**Figure 2C**). AMF inoculation had a negative effect on root volume with respect to the control (**Figure 2C**).

The control with fertilization in both chili bell pepper cultivars showed the highest total dry weight, as observed in **Figures 1D** and **2D**. In Bell Pepper, the treatment with the AMF H1 consortium resulted in a higher total dry weight ($p = 0.05$) than the other treatments, except for the fertilized control; something very similar was observed for jalapeño bell pepper: with H1, a significantly higher total dry weight was obtained than with the other treatments ($p = 0.05$), except for the fertilized control and the one inoculated with the P61 bacteria. Regarding inoculation with bacteria, it is observed in **Figure 1D** that Bell Pepper plants inoculated with R44 were the only ones of that cultivar that showed higher total dry weight than the unfertilized control ($p \leq 0.05$), while in jalapeño bell pepper this occurred with those inoculated with P61 ($p \leq 0.05$).

Figures 3 and **4** show the foliar parameters evaluated in this research. It is observed that the fertilized control showed a higher number of leaves in both chili bell pepper cultivars (**Figures 3A** and **4A**). In Bell Pepper, only the treatments inoculated with A46, Ols-Sf5 and R44 bacteria were in this respect superior to the control ($p = 0.05$) (**Figure 3A**). On the other hand, in jalapeño bell pepper plants, with all the bacteria tested, a greater number of leaves was obtained than in the control (**Figure 4A**). Regarding the treatments with AMF, H2 and H3 inoculated on Bell Pepper plants led to a significant increase ($p = 0.05$) in the number of leaves with respect to the control (**Figure 3A**), while in jalapeño bell pepper the 3 mycorrhizal inocula, H1, H2 and H3, were associated with a higher number of leaves than in the control (**Figure 4A**).

In both chili bell pepper cultivars, the control with fertilization achieved the greatest leaf area, as shown in **Figures 3B** and **4B** ($p = 0.05$). As for the biological treatments, it is noticed in **Figure 3B** that the inoculation of Bell Pepper plants with bacteria A46, P61 and R44 led to a higher leaf area compared to the control ($p = 0.05$), while with the application of the 3 AMF treatments (H1, H2 and H3), higher leaf areas were obtained with respect to the control. In the case of jalapeño bell pepper plants, those inoculated with P61 and R44 bacteria showed significantly positive effects ($p = 0.05$) on leaf area compared to the control (**Figure 4B**).

The fertilized control also showed higher leaf dry weight in both chili bell pepper cultivars, as shown in **Figures 3C** and **4C**. In Bell Pepper, the only bacterial treatment that led to a significant ($p = 0.05$) increase in this parameter was that of those inoculated with R44, which outperformed the other PGPR treatments and the control (**Figure 3C**). In jalapeño bell pepper, those inoculated with P61 had the highest leaf dry weight compared to the other bacterial treatments and the control (**Figure 4C**). Regarding AMF performance, in Bell Pepper plants only those inoculated with H3 showed a higher leaf dry weight ($p = 0.05$) than that observed for the other

treatments, except for the fertilized control (**Figure 3C**). In jalapeño bell pepper plants, those inoculated with the H1 consortium were superior in leaf dry weight (p = 0.05) to the other treatments, except for H3 and the fertilized control (**Figure 4C**).

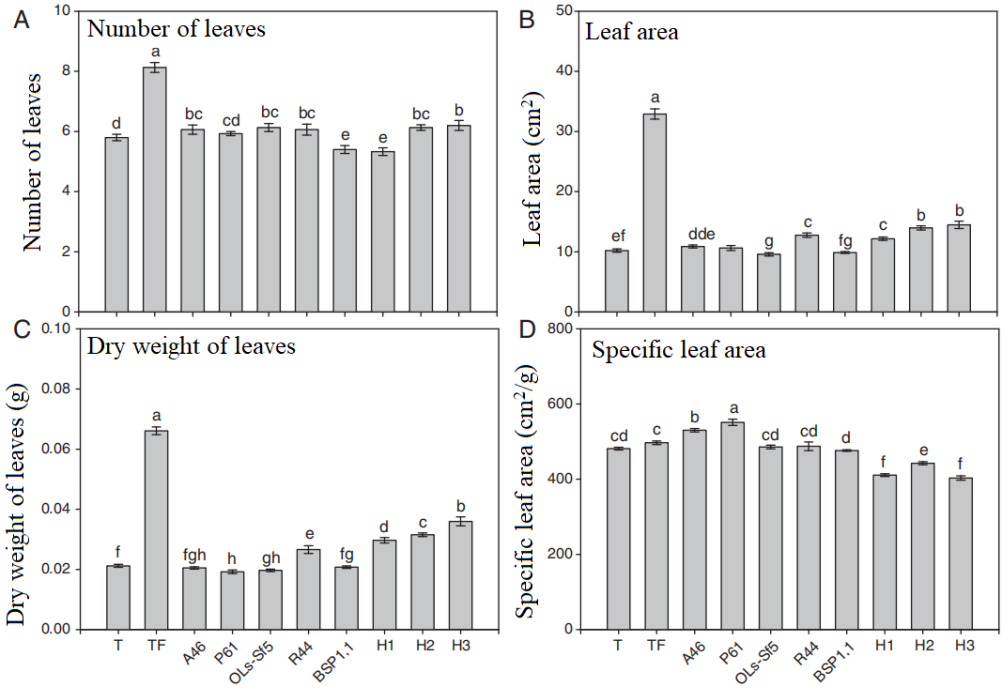

Figure 3. Number of leaves (**A**), leaf area (**B**), Dry weight of leaves (**C**) and Specific leaf area (**D**) of Bell Pepper plants after 70 days. Means are shown standard error, n = 15. Identical letters above the bars indicate no significant difference (LSD, α = 0.05). A46: *Pseudomonas tolaasii*; P61: *Pseudomomas tolaasii*; OLs-Sf5: *Pseudomomas* sp. R44: *Bacillus pumilus*; BSP1.1: *Paenibacillus* sp.; H1: *Funneliformis aff. geosporum* and *Claroideoglomus* spp; H2: *Rhizophagus intraradices*; H3: *Rhizophagus fasciculatus*, *Glomus* sp, and *Archaeospora* sp.; T: absolute control; TF: fertilized control.

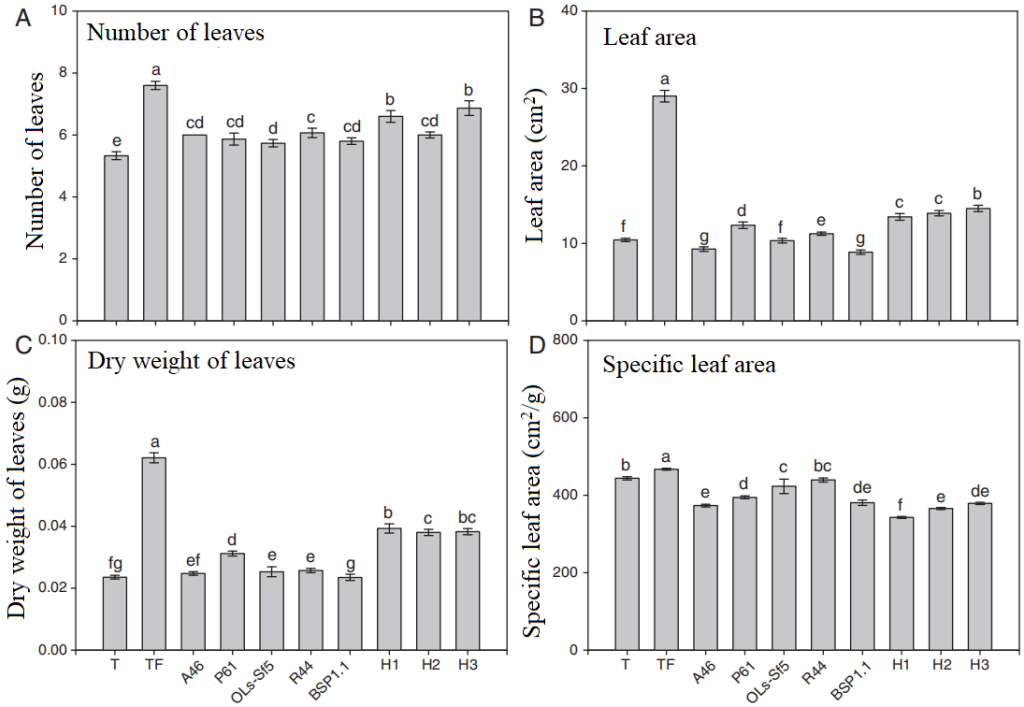

Figure 4. Number of leaves (**A**), leaf area (**B**), Dry weight of leaves (**C**), Specific leaf area (**D**) of Jalapeño bell pepper plants after 70 days. Means are shown standard error, n = 15. Identical letters above the bars indicate no significant difference (LSD, α = 0.05). A46: *Pseudomonas tolaasii*; P61: *Pseudomomas tolaasii*; OLs-Sf5: *Pseudomomas* sp. R44: *Bacillus pumilus*; BSP1.1: *Paenibacillus* sp.; H1: *Funneliformis aff. geosporum* and *Claroideoglomus* spp.; H2: *Rhizophagus intraradices*; H3: *Rhizophagus fasciculatus*, *Glomus* sp. and *Archaeospora* sp.; T: absolute control; TF: fertilized control.

Figure 3D shows that Bell Pepper plants inoculated with P61 bacteria showed greater specific leaf area compared to the other treatments (p = 0.05), while the fertilized control showed greater specific leaf area compared to the other treatments at the same level of statistical signification (**Figure 4D**). As for AMF inoculation, no positive effects on this parameter were observed in comparison with the control or the fertilized control in both Chile cultivars (**Figures 3** and **4**).

Finally, **Figure 5** shows the effect of treatments on photosystem ii (PSII) photosynthetic efficiency (Fv/Fo). It can be seen that the application of mineral fertilizer led to a significant increase with respect to the rest of the treatments. In the case of Bell Pepper, no significant differences (p = 0.05) were observed between the different PGPRs, and the Fv/Fo values were mostly significantly higher than those obtained with AMF inoculation (**Figure 5A**). In jalapeño bell pepper, Fv/Fo values did not show significative differences (p = 0.05) among the treatments inoculated with the bacteria with respect to the control, and the lowest value was obtained in the treatment inoculated with *R. intraradices* (H2), whose Fv/Fo was even significatively lower than that of the control (**Figure 5B**).

Total mycorrhizal colonization showed significant differences (p = 0.05) between treatments inoculated with mycorrhizal fungi on both Chile cultivars. The H3 consortium was more infective on both cultivars: 47.2% on Bell Pepper and 42.3% on jalapeño bell pepper; the H2 inoculum showed 32.8% colonization on jalapeño bell pepper. Inoculum H1 showed low infectivity (less than 5%) on both cultivars. The presence of arbuscules was not observed in any cultivar. The presence of vesicles in Bell Pepper cultivar was only observed when inoculated with H3 (19.3%) and in jalapeño, H2 and H3 treatments produced 17.4 and 27.8% vesicles, respectively (**Table 1**).

Table 1. Colonization of AMF in chili bell pepper (*Capsicum annuum*) Bell Pepper and jalapeño plants

Cultivate	AMF[a]	Total colonization (%)	Arbuscules (%)	Vesicles (%)
Bell Pepper	T	0 c	0	0
	H1	3.5 b	0	0
	H2	4.5 b	0	0
	H3	47.2 a	0	19.3
Mexico	T	0 c	0	0
	H1	4.6 c	0	0
	H2	32.8 b	0	17.4
	H3	42.3 a	0	27.8

H1: *Funneliformis aff. geosporum* and *Claroideoglomus* spp.; H2: *Rhizophagus intraradices*; H3: *Rhizophagus fasciculatus*, *Glomus* sp. and *Archaeospora* sp.; T: absolute control. [a] Mycorrhizal colonization was not found in the treatments inoculated with bacteria, so these are not shown in the table. Equal letters following means indicate no significant difference (LSD, α = 0.05).

4. Discussion

The bacterial strains that showed the greatest effect on plant height, leaf area, stem diameter and root volume with respect to the unfertilized control in both chili cultivars were P61 and R44, while the most effective AMF in plant height, stem diameter and plant dry weight corresponded to the H1 and H3 consortia. These results reflect the relevance of microorganisms in the development of seedlings of horticultural interest, as they would allow reducing the demand for agricultural inputs, especially chemical fertilizers[32]. Planting and transplanting practices are common in agricultural systems, hence it is important to produce healthy seedlings in order to achieve high yields after transplanting. It should be noted that the use of labor in the production of seedlings in seedbeds or during transplanting is mandatory, so that this stage can be used to inoculate with beneficial microorganisms. In direct field production this would be more costly and the microorganisms could have greater difficulty in colonizing the rhizosphere of the plant[33,34].

The benefits of the bacterial strains used in this experiment in the promotion of plant growth have been documented in different crops such as turnip (Brassica napus), Bell Pepper (*Capsicum annum* L.), cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*). The growth-promoting

mechanisms of these bacteria are related to siderophore production, phosphate solubilization and indole synthesis[24,35–39].

In the case of AMF, their favorable effects have been reported in Bell Pepper (*Capsicum annum* L.) and oat (*Avena sativa*) plants, among them the production of aerial and root biomass, some physiological parameters and the accumulation of phosphorus[38,40] stand out. Such results are related to the findings of the present study in both chili varieties. AMF promote plant growth due to a greater absorption of nutrients such as phosphorus and nitrogen, among others[38,41–44].

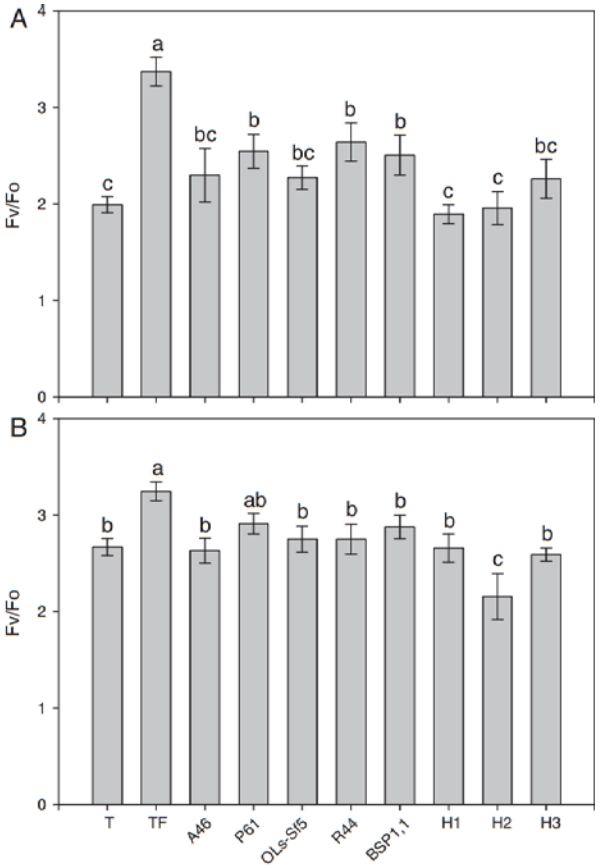

Figure 5. Photochemical efficiency of photosystem ii expressed as Fv/Fo in Bell Pepper (**A**) and jalapeño (**B**) chili pepper plants after 70 days. Means ± standard error is shown, n = 8. Identical letters above the bars indicate no significant difference (LSD, α = 0.05). A46: *Pseudomonas tolaasii*; P61: *Pseudomomas tolaasii*; OLs-Sf5: *Pseudomomas* sp. R44: *Bacillus pumilus*; BSP1.1: *Paenibacillus* sp.; H1: *Funneliformis aff. geosporum* and *Claroideoglomus* spp.; H2: *Rhizophagus intraradices*; H3: *Rhizophagus fasciculatus*, *Glomus* sp, and *Archaeospora* sp.; T: absolute control; TF: fertilized control.

Significant differences were obtained in the C. annum cultivars tested here when inoculated with strains P61 (*P. tolaasii*) and R44 (*B. pumilus*): for most of the variables evaluated, positive effects were observed with these treatments. Kang *et al.*[45] reported similar results when inoculating 2 endophytic strains (*Pseudomomas* sp. and *Pantoea* sp.) on bell pepper seedlings, these strains promoted growth by 16.6 and 17.2%, respectively, and total fresh weight by 27.7 and 15.3%, respectively. There are no reports on inoculation with *P. tolaasii* and *B. pumilus* in these chili bell pepper varieties; however, the favorable effect of inoculation with PGPR on growth and other characteristics related to seedling quality has been studied by Diaz *et al.*[46] and by Brutti *et al.*[47] in tomato and lettuce.

Overall, in the 2 cultivars of *C. annuum*, a greater positive effect on growth parameters (height, stem diameter, leaf area, and leaf and total dry weight) was observed for AMF inoculation compared to CPB inoculation. This effect is mainly attributed to the physiological activity of AMF, which favors plant growth, development and vigor, as discussed by Smith and Smith[48].

Root volume, on the other hand, showed a greater response to bacterial inoculation than to AMF inoculation. This effect may be related to the fact that, in mycorrhizal plants, the fungal hyphae explore the soil more effectively and help to absorb and assimilate nutrients in the plants, i.e., they act as an extension of the roots, so that the plant does not require more root development to absorb and assimilate nutrients[49]. Aguirre-Medina and Kohashi-Shibata[50] found that inoculation with AMF increased the development of the aerial part, but caused a lower root dry weight in bean seedlings. On the other hand, Soti *et al.*[51] observed a similar response in *Lygodium microphyllum*, obtaining a negative correlation between mycorrhization and root growth, without affecting the development of the aerial part of the plant. The above indicates that the root growth response of a mycotrophic plant could be mediated by AMF.

The measurement of the photochemical efficiency of PSII based on chlorophyll fluorescence is an effective, non-invasive technique to detect damage to PSII7, and the parameter Fv/Fo indicates the potential photosynthetic capacity of PSII[52]. In both chili cultivars, control plants with fertilization showed higher Fv/Fo values than treatments

inoculated with PGPR or with AMF; this is because the seedling, having all nutrients proportionally, does not suffer stress and this reflects in high Fv/Fo readings[53] Russo and Perkins-Veazie[34] found that there were no responses in terms of chlorophyll in Bell Pepper seedlings inoculated with either PGPR or AMF, either alone or in combination. In contrast, the unfertilized control showed the lowest Fv/Fo values; this is attributed to the limited availability of nutrients in the seedling root ball, which may cause nutrient deficiency stress, since photosynthetic efficiency is related to a decrease in leaf nitrogen content, chlorophyll content, and leaf area[54]. On the other hand, plants with AMF showed values of Fv/Fo similar to the control, but lower than the treatments inoculated with PGPR and the control with fertilization. This may be related to a greater consumption of photoassimilates by the symbiont fungus, which would cause stress to the plant that would be reflected in low Fv/Fo values[48]; however, some studies show that AMF accelerate photosynthetic activity in the host plant[55,56]. In this case, AMF, by promoting plant growth, could have depleted nutrients in the substrate; this would lead to nutrient stress in the seedlings at the final of the experiment, at which time low Fv/Fo readings were present, indicating plant stress[52]. This situation did not occur in the fertilized plants, in which the highest Fv/Fo values were recorded.

Growth promotion by AMF or GCPVB in both Chile cultivars is related to increased growth in the aerial part, which may be the result of various mechanisms, such as nitrogen fixation and phosphate solubilization, or the production of different phytohormones (indoleacetic acid, gibberellic acid, and cytokinins), which favor leaf expansion, which in turn favors resource utilization[57,58].

The increase in the photochemical efficiency of PSII, represented with the parameter Fv/Fo, is related to growth promotion in both chili cultivars, mainly due to the inoculation of strains P61, R44 and BSP1.1 with respect to the absolute control, indicating a benefit in the PSII of the plants[59]. Therefore, the fluorescence of chlorofila has value in the early diagnosis of plant vitality or vigor, even before it is diagnosed by the naked eye[60].

Mycorrhizal colonization was low with the H1 consortium and H2 inoculum in Bell Pepper and with the H1 consortium in jalapeño bell pepper, however; there was a favorable response to AMF in plant height, stem diameter, total dry weight, leaf number and leaf area, indicating that the extent of mycorrhizal colonization is not always a clear indicator of the potential benefit it may represent for its host plant[61,62].

AMF H1 and H3 and PGPR P61 and R44 increased the growth of jalapeño bell pepper and Bell Pepper seedlings. Despite low mycorrhizal colonization, AMF produced favorable effects on plant height, stem diameter and plant dry weight. In the case of PGPRs, the main benefits were related to plant height, leaf area, stem diameter and root volume with respect to the control without fertilization. In addition, the PGPRs produced an increase in Fv/Fo compared to the unfertilized control, which is related to the increased photosynthetic capacity of PSII. However, the use of PGPR or AMF alone is not sufficient to obtain benefits comparable to those achieved with chemical fertilization. New experiments aimed at finding optimal doses of chemical fertilization, compatible with the physiological activity of PGPRs and AMFs in plants, are required.

These microorganisms can be used to reinforce the development of Mexico bell pepper and Bell Pepper seedlings, so that these reach the field with more adaptive faculties against the different types of stress that could occur after transplanting. Although in this research work the interaction between the two different types of microorganisms was not studied, the possibility of a synergistic effect between them is not ruled out. In this regard, there are studies that demonstrate a positive effect on the promotion of plant growth when PGPR and AMF are mixed[63,64].

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Conflict of interest

The authors declare that there is no conflict of interest in this scientific manuscript.

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