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Plant Growth Promoting Rhizobacteria (PGPR) Isolated from an Arid Soil in Saudi Arabia Improve Maize Growth

Abdelazize Eljiati[1*], Yassine Elmaati[2], and Hammou Ouchaou[2]

[1] National Centre for Palms & Dates, Al Dahi, Hittin, Riyadh 13512, Saudi Arabia; [2] R&D Department, YALA Laboratory, Yousef Abdul Latif and Sons Agriculture Ltd. (YALA) Company, Qassim, Saudi Arabia

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Abstract

The rhizosphere represents the main source of bacteria commonly referred to as rhizobacteria. Such beneficial rhizobacteria with plant-beneficial activities are generally defined as plant growth promoting rhizobacteria (PGPR). The aim of this study was to investigate the ability of native rhizobacteria (PGPR) isolated from an arid soil of date palm in Al-Qassim region, Saudi Arabia, to enhance plants growth. Maize (*Zea mays* L.) was used as model crop for this research. Maize seedlings roots were inoculated with *Bacillus* and *Enterobacter* bacteria. The seedlings showed significant increases in stem, leaf, and root growth. The maximal shoot lengths were obtained with strain (I2: Bacillus cereus) (95.41 cm) with an increase of 33.45 % compared to uninoculated control seedlings. The three isolates I2: *Bacillus cereus*, AZS2: *Bacillus subtilis* and commercial strain AZB: *Azospirillum brasilense*) caused a highly significant increase in the total number of leaves ranging from 10.9% to 12.7% compared to the uninoculated controls. Seedlings inoculated with AZS2: *Bacillus subtilis* strain exhibited the highest aerial dry biomasses with an improvement of more than 85 % (30.76 g) compared with uninoculated control plants and more than 62 % compared to uninoculated NaCl control plants. The inoculation treatment with I2: *Bacillus cereus* strain induced an improvement of more than 65 % (27.44 g) over uninoculated control and more than 45 % over uninoculated NaCl control. The strain AZS2: *Bacillus subtilis* produced the highest root dry weights, in comparison to other isolates and induced an improvement of 30.17% (26.06 g) compared to uninoculated control plants and 24.09% compared to uninoculated plants (NaCl control). The most effective rhizobacterial treatment in the dry biomasses of whole seedling (aerial dry biomass and root dry biomass) is AZS2: *Bacillus subtilis* strain which induced an improvement of 55% (56.83 g) compared to uninoculated plants (control) and 42% compared to uninoculated plants (NaCl control). The most important production of kernels was recorded with AZS2: *Bacillus subtilis* strain. Therefore, these findings suggested that the use of PGPR strains as inoculant biofertilizers might be beneficial for crop production cultivation especially in arid and semi-arid regions.

Keywords: Plant growth promoting rhizobacteria (PGPR), *Bacillus subtilis, Bacillus cereus, Enterobacter ludwigii*, inoculation, *Zea mays* L.

[*] Corresponding author: a.eljiati@gmail.com

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Introduction

Bacteria that can aggressively colonize the rhizosphere or plant roots or both and promote growth and yield of plants are referred to as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1989). These rhizosphere bacteria enhance crop growth and yield directly, either by promoting nutrition, for example, by phosphate (Hayat et al., 2010; Sharma et al., 2007; Das et al., 2003), and potassium solubilization (Wang et al, 2020; Han et al., 2006) and ammonia production (Mukhtar et al., 2020; Ahmad et al., 2008; Joseph et al., 2007) or by synthesizing metabolites with great agricultural interest such as plant growth regulators and siderophores (Tian et al., 2009; Arkhipova et al., 2005; Barazani et Friedman, 2001). They can also promote growth indirectly, acting as bio-controlling agents for suppression of growth of soil borne phytopathogen microorganisms and as stimulator of other beneficial organisms for the plant (Abbasi et al., 2011; Bhattacharyya and Jha, 2011). PGPR improve soil structure and bioremediate polluted soils by sequestering toxic heavy metal and degrading xenobiotic compounds (Ahemad et al., 2012; Braud et al., 2009). Depending on their beneficial roles in the rhizosphere, PGPR have been classified as biofertilizers, phytostimulators, rhizoremediators and biopesticides (Martínez-Viveros et al., 2010). Consequently, the application of these beneficial microorganisms as bioinoculants appears as an ecological friendly biotechnological tool (Dimpka et al., 2009) to alleviate detrimental effects of intensive farming practices that are using synthetic fertilizers and pesticides without caring about environmental problems and soil health (Elkoca et al., 2010). Numerous laboratory, greenhouse and field studies are available on the screening of PGPR for their multiple plant growth promoting activities (Wang et al, 2020; Gouda et al., 2018; Oteino et al., 2015; Hayat et al., 2010; Joseph et al., 2007) and utilization of PGPR-based products in agricultural crop production systems (Yadav et al., 2017; Cakmakci et al., 2006). These products are mainly applied as seed treatment, soil amendment, or soil drench at the time of sowing or immediately after transplantation, to facilitate better nutrient uptake, greater production of growth hormone and beneficial phytochemicals in crops leading to higher crops yield and quality (Kloepper et al., 2004). PGPR activity has been reported in strains belonging to a several genera, such as Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Rhizobium, Bradyrhizobium, Alcaligens, Arthobacter, Burkholderia, Bacillus, Serratia and Xanthomonas (Verma et al., 2013; Karnwal 2009; Patten and Glick 1996; Glick, 1995; Kloepper et al., 1989). Pseudomonas and Bacillus spp. have been the most studied bacteria for their plant growth promotion (PGP) activity and ability to produce beneficial substances (Kejela et al., 2016; Pham et al., 2017). Isolation of native strains adapted to the arid environment may contribute to formulation of inoculants suitable for use in local crops, as they are adapted to the environment and can be, thereby more competent than imported microbial strains. The positive impact of PGPR has been studied in annual crops like wheat (Bashan, 1986), soybeans (Cattelan et al., 1999), beans (Jarak et al., 2012) and corn (Di Salvo et al., 2018; Ullah et al., 2014) in several ways.

Therefore, this study was designed to select effective strains from a series of of native rhizobacteria (PGPR) isolated in an arid area soil of date palm in Al-Qassim region, Saudi Arabia, by maize (*Zea mays* L.) growth promotion assay under greenhouse conditions. These native strains are also compared with a commercial microbial strain, as a positive control. Therefore, the application of the selected effective PGPR strains as microbial inoculants for crops would significantly promote their sustainable production in arid conditions and reduce the use of inorganic fertilizers and pesticides, which often pollute the environment.

Materials and Methods

Bacterial Inoculants

The rhizobacterial strains (I2: *Bacillus cereus*, AZS2: *Bacillus subtilis*, AZA2: *Enterobacter ludwigii* and PSA1: *Enterobacter ludwigii*) used in this study were previously isolated from an arid area soil in Qassim province, Saudi Arabia (Elmaati et al., 2020). These bacterial strains were characterized and selected based on their plant growth promoting traits, comprising very good phosphate and potassium solubilization and ammonium production. A commercial strain (AZB; *Azospirillum brasilense*) was used as a positive control to compare it with these native strains.

Inoculation of Maize Plants by PGPR Strains

To prepare the inoculum for each rhizobacterial strain, pure cultures were grown in nutrient agar (R2A Agar medium). After 48 hours of incubation at 25 °C, a single colony from each strain was transferred into a 200 ml sterilized Erlenmeyer flask, containing sterilized 102 medium broth (= LMG 1089 medium) with the following composition (g L-1): Sucrose, 20.0; Casein hydrolyzate, 16.0; Yeast extract, 8.0; KH2PO4, 4.0; MgSO4 x 7 H2O, 0.30, and grown aerobically for 4-5 days on a rotating shaker (150 rpm) at 32 °C, to obtain a final concentration of 109 CFU ml-1. After incubation, bacterial growth is estimated by measuring the absorbance of the culture at 600 nm. To wash the bacteria, bacterial cells were centrifuged at 3000 rpm for 10 min in 15 ml tubes. The supernatant was then discarded and the pellet was washed once with 5 ml of sterile NaCl solution (Physiologic Sterile Water (0.85%)) and finally resuspended in 200 ml of the same solution.

Maize (*Zea mays* L.) was used as the test plant for the inoculation in this experiment. Seeds of a homogeneous variety were surface sterilized to eliminate all kinds of contamination according to the method of Götz et al. (2006): The seeds were immersed for 1 min in ethanol (70%) with gentle agitation. They are then put back into 12% diluted sodium hypochlorite solution containing three drops of wetting agent (Tween 20) for 15 minutes. To get rid of the chlorine, the seeds were rinsed several times with sterile distilled water.

Sterilized seeds were sown in alveolar plates containing

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autoclaved (1: 1 v / v) mixture of peat and vermiculite respectively. They were then placed in a greenhouse at a natural photoperiod (at a temperature of 28 to 40 °C) and are regularly irrigated.

Twenty days after germination, the roots of maize seedlings were dipped into the inocula for 2 h at 25 °C. Control seedlings were divided into two groups; the first is dipped into sterile NaCl solution as the bacteria washing procedure containing this solution, while the second is dipped into sterile distilled water. Each treated seedling was planted in a disinfected and labeled plastic pot (8 L) containing autoclaved (1/1: v / v) mixture of soil and vermiculite respectively. Pots were then placed in a greenhouse at a natural photoperiod (at a temperature of 28 to 40 °C). The experiment was carried out in a completely randomized block design with 5 replications for each treatment. Seedlings were irrigated with 300 ml of well water every two days to maintain at field capacity and received no fertilizers.

Estimation of Some Agro Morphological Parameters of Plants

To evaluate the response to rhizobacterial inoculation, during the experiment, a daily monitoring of the evolution of the growth of the maize plants was done after the application of the inoculum for a period of 90 days. The height from the collar (size of the aerial part) and the number of leaves are the main growth parameters used in this study. These two growth parameters were measured at the start and every ten days during the 3 months of treatment. Plant height was determined by measuring from the plant's base to the top of the newest fully developed leaf.

At the end of the experiment, the shoots and roots of each plant were put in paper bags and dried in an oven at 65°C for 72 hours (Sfairi, 2013) to report the total dry biomass (shoot and root dry biomass).

Statistical Data Analysis

The mean value of each treatment, as well as the corresponding standard deviation, were calculated using the data of all the replicates carried out. The data obtained was analyzed statistically using R 3.2.0 and multivariate analyses were performed using R language (Dray & Dufour, 2007; R Development Core Team, 2011).

Results

Effect of Rhizobacterial Inoculation on Shoot Length

The rhizobacterial isolates (AZB, AZS2, I2, PSA1, and AZA2) significantly affected the shoot length of maize seedlings. Results reveal that PGPR promoted an increase in shoot length over un-inoculated (control) (Figure 1 - A and B). The maximal lengths of the maize seedlings were obtained with strain (I2) (95.41 cm) with an increase of 33.45 % compared to un-inoculated (controls). Treatment of maize seedlings with strain (I2) showed a significant increase in shoot length improvement rates compared to other strains including the commercial strain (AZB). There was no significant difference in shoot length between the two controls.

Effect of Rhizobacterial Inoculation on Leaf Numbers

From 1 to 38 days after emergence (Figure 2A), the number of leaves of maize plants increased linearly in all treatments. These results were identical for all the rhizobacterial isolates and the controls. However, we noted the beginning of the stability of the number of leaves between the 40^{th} and the last days of the cycle in some plants.

The effects of different rhizobacterial isolates were significant on the total number of leaves of maize, compared with control. Application of AZS2 isolate to maize seedlings recorded non-significantly higher number of leaves, compared with AZB and I2, and all these three isolates were comparatively more effective than rest of the isolates and the two un-inoculated control. The three effective isolates caused a highly significant increase in the total number of leaves ranging from 10.9% to 12.7% compared to the un-inoculated control. No significant difference was observed between the two isolates (PSA1 and AZA2) and the control with NaCl (Figure 2B).

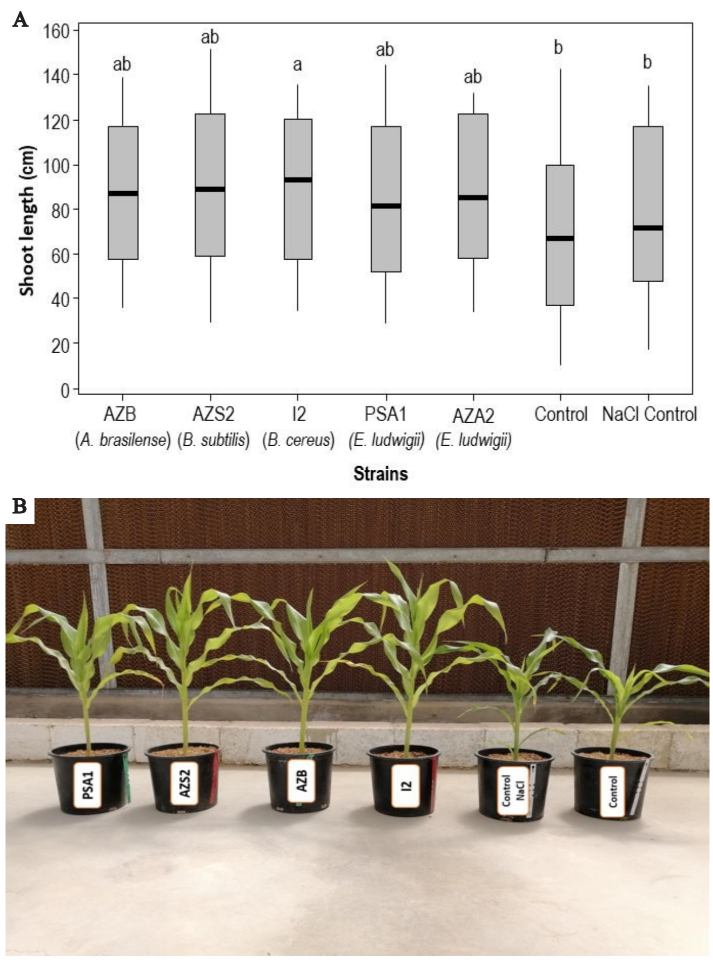

Figure 1. (A) Effect of rhizobacterial inoculation on shoot length (B) Height growth of maize seedlings after 50 days of cultivation under the effect of applied rhizobacterial strains.

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Effect of Rhizobacterial Inoculation on Leaf Desiccation

During the period of evolution of the different foliar levels of the maize seedlings, we noticed that the leaves of the base wither and turn brown, by a yellowing and drying which begins with the end of the leaves and extends thereafter, until they dry out completely. The number of dried older leaves was recorded for each plant. At the end of the experiment, the results of the analysis of variance relating to this parameter show that there is no statistically significant difference between the rhizobacteria and the controls without bacteria (Figure 3).

Effect of Rhizobacterial Inoculation on Aerial Dry Biomass

The analysis of variance relative to the aerial dry biomasses of maize seedling shows that there is a highly significant difference between the different strains and the un-inoculated control and un-inoculated control NaCl control (with a total aerial dry biomass which marked a rate of 16.59 g and 18.88 g respectively). Seedlings inoculated with AZS2 strain exhibited the highest aerial dry biomasses with an improvement of more than 85 % (30.76 g) compared with un-inoculated control plants and more than 62 % compared to un-inoculated NaCl control plants. The inoculation treatment with I2 strain

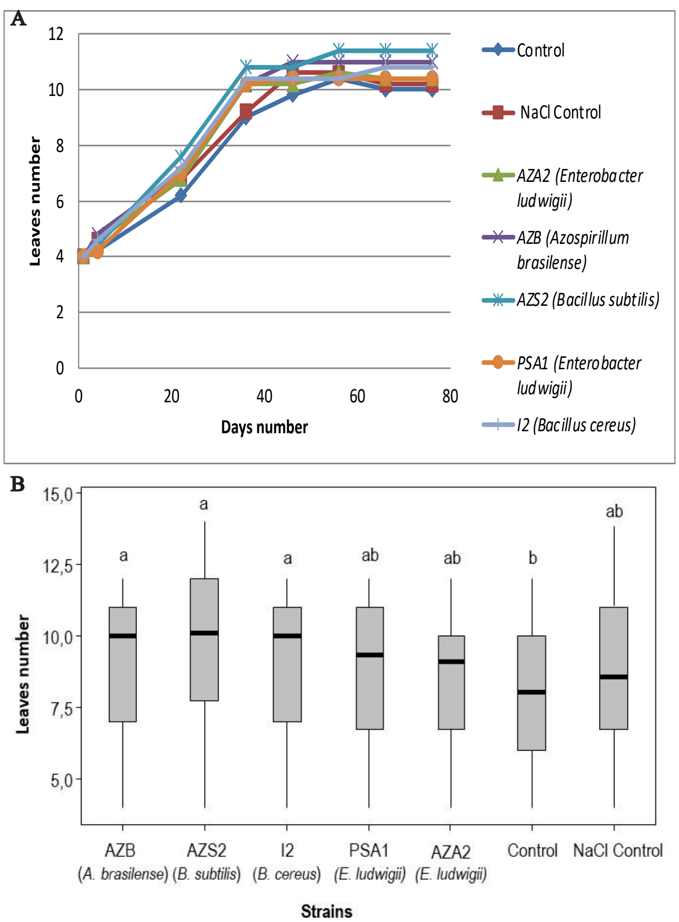

Figure 2. (A) Evolutionary trend of the adjusted mean of the number of maize leaves (B) Effect of rhizobacterial inoculation on leaf numbers.

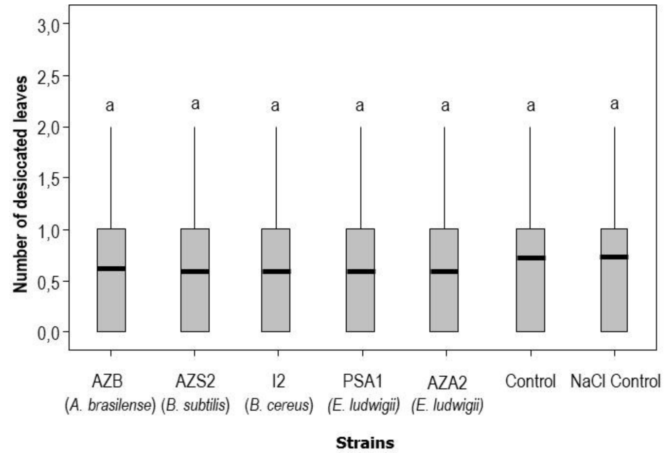

Figure 3. Effect of rhizobacterial inoculation on leaf desiccation.

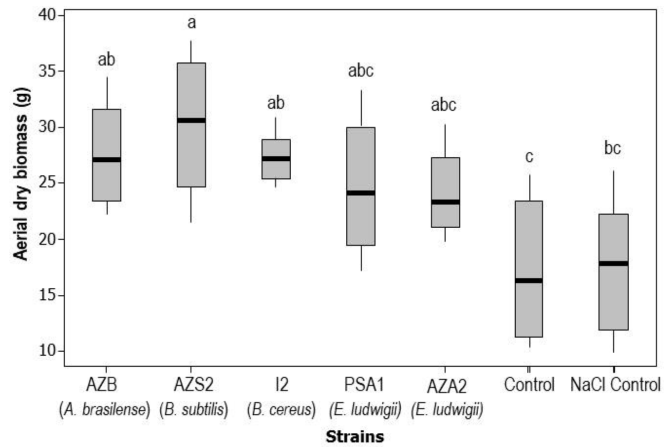

Figure 4. Effect of rhizobacterial inoculation on aerial dry biomass

induced an improvement of more than 65 % (27.44g) over un-inoculated control and more than 45 % over un-inoculated NaCl control. On the other hand, the commercial strain (AZB) showed an improvement of 64.98% (27.37 g) compared to untreated control and 44.97% compared to untreated NaCl control. The others two strains (PSA1 and AZA2) are characterized by their lowest significant effect on aerial dry biomass per comparison to the both control plants (control and NaCl control) (up to 38 % increase) (Figure 4).

Effect of Rhizobacterial Inoculation on Root Dry Biomass

The effect of the five rhizobacterial strains on the root dry biomasses of maize plants is illustrated in (Figure 5A). All the tested strains were significantly improved the root dry biomasses in comparison with controls (control and NaCl control), which scored a rate of 20.02 g and 21.00 g respectively. The strain AZS2 produced the highest root dry weights, in comparison to other isolates and induced an improvement of 30.17% (26.06 g) compared to un-inoculated plants (control) and 24.09% compared to un-inoculated plants (NaCl control). While, in comparison with controls, the inoculation with strains I2, PSA1 and AZB showed a significant difference with improvement rates ranging from 16.2% to 19.35%.

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It is observed from the results (Figure 5B) that the rhizobacterial strains caused greater increase in root system of maize plants as compared with controls (control and NaCl control). Indeed, the highest root lengths was recorded with the inoculation of AZS2 and I2 strains), in comparison to other strains.

Effect of Rhizobacterial Inoculation on Whole Seedling Dry Biomass

A very highly significant improvement rate in the dry biomasses of whole seedling (aerial dry biomass and root dry biomass) inoculated with the rhizobacterial strains is recorded compared to the un-inoculated plants (control and NaCl control), which scored a level of 36.62 g and 39.94 g respectively. The most effective rhizobacterial treatment is AZS2 strain which induced an improvement of 55% (56.83 g) compared to un-inoculated plants (control) and 42% compared to un-inoculated plants (NaCl control). Analysis of the variance applied to dry biomasses of whole seedling indicated that there was no significant difference between other strains (I2, AZA2, PSA1 and AZB), but they induced an increase for this parameter compared to the un-inoculated control (Figure 6).

Effect of Rhizobacterial Inoculation on Kernel Numbers

All the inoculated treatments proved statistically superior over un-inoculated control in improving number of kernels. No significant difference was observed between the four bacterial strains AZB, AZS2, I2 and PSA1. However, the most important production of kernels was recorded with AZS2 strain. While total kernels number was significantly increased with these four strains as compared to AZA2 strain and compared to un-inoculated control (Figure 7).

Discussion

Plant Growth Promoting Rhizobacteria (PGPR) are free-living microbes that live on or around the roots (Kloepper et al., 1989) and promote plant growth and yield (Wu et al., 2005). Native rhizobacteria (PGPR) isolated in arid area soil of date palm in Al-Qassim region, Saudi Arabia, were used in this study to constitute the rhizobacterial inoculum, which was then used to inoculate maize (*Zea mays* L.) seedlings. These strains exhibited significant plant growth promoting attributes in vitro tests and selected from previous screening experiments (Elmaati 2020). In addition to its nutritional and

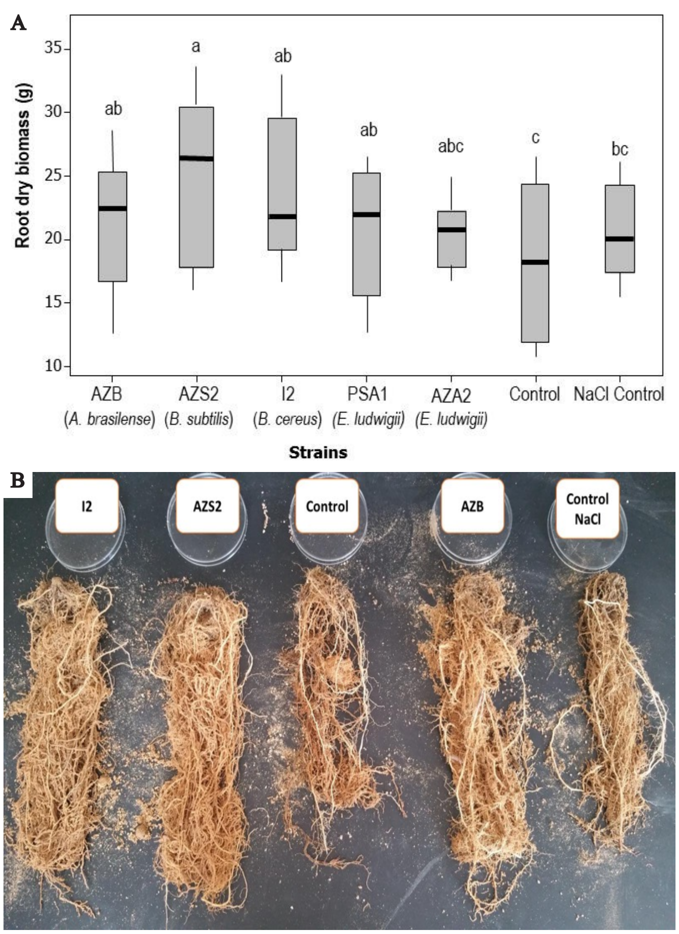

Figure 5. (A) Effect of rhizobacterial inoculation on root dry biomass (B) Effect of rhizobacterial inoculation on root length of maize seedlings

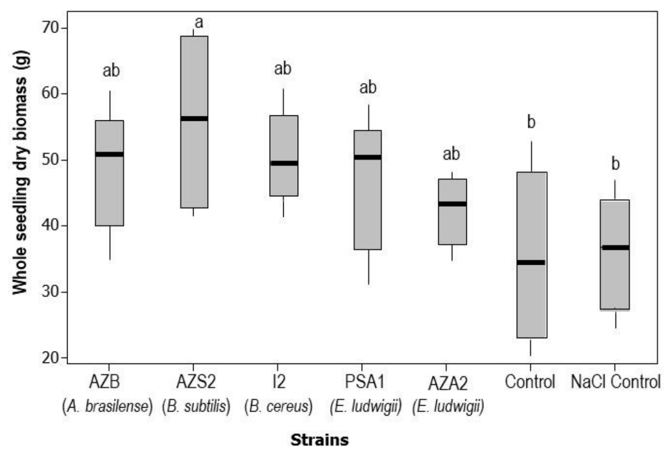

Figure 6. Effect of rhizobacterial inoculation on whole seedling dry biomass.

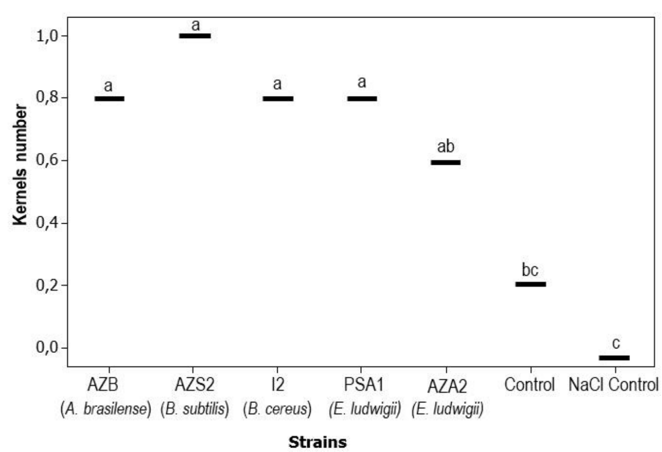

Figure 7. Effect of rhizobacterial inoculation on kernel numbers.

economic importance, maize has been a keystone model organism for basic and applied research in plant biology (Strable and Scanlon, 2009).

Overall, the results of the study of the growth of maize seedlings treated with the various rhizobacterial strains enabled us to conclude that the efficacy of different strains for growth-promoting of maize was variable. Indeed, the investigated PGPR strains in this work showed positive PGP traits. These potentialities seem playing an effective role for the plant in helping it to better absorb nutrients. PGPR have long been known to promote growth when added to seeds, roots or tubers in a wide range of plant species (Kloepper et al., 1980), increasing both growth and yield (Wu et al., 2005) by improving the concentration of nutrients in the host plant (Canbolat et al., 2006).

Several studies have reported that inoculation of maize plants with PGPR strains caused significant increase in plant height, plant dry weight, stem diameter, root length and weight, yield, number of leaves and leaf area, and plant nutrient uptake of N, P, K, Fe, Zn, Mn and Cu (Yazdani et al., 2009; Jarak et al., 2012; Gholami et al., 2012; Calvo et al., 2017). According to our results maize seedlings inoculated by dipping the roots in bacterial suspensions showed a statistically significant improvement in the growth parameters studied as compared to treatments without inoculation. Interestingly, the plant height, number of leaves, shoot dry weight, root dry weight and kernels number were significantly higher with plants treated with AZS2 strain (*Bacillus subtilis*) followed by I2 strain (*Bacillus cereus*) in comparison with un-inoculated control and with other strains including the commercial strain (AZB, *Azospirillum brasilense*). This is consistent with previous studies, which demonstrated that plant growth-promoting activities of Bacillus spp. are well characterized as evidenced by increased growth of roots, shoots, and leaves as well as enhanced yields. In this context, increased plant height and shoot biomass of Arabidopsis, corn, and tomato under greenhouse conditions have been reported by inoculating with four isolated Bacillus strains from rainforest soils (Huang et al., 2015). Results obtained by Hassan (2017) report that *B. cereus* Tp.1B and *B. subtilis* Tp.6B strains significantly increased root length and root weight in maize compared to controls. Co-inoculation of Bacillus spp with other PGPR strains reduces phosphorus demand by 50% without affecting maize yield (Yazdani et al., 2009). Moreover, Ferreira et al., 2018 reported that *Bacillus subtilis* promotes positive influence on plant growth of maize plants under normal conditions (without salinity). *Bacillus subtilis* strain was the most effective in promoting nitrogen accumulation and, therefore, increased chlorophyll content in maize (Aquino et al., 2019; Almaghrabi et al. al., 2014). When tomato seeds were treated with *Bacillus subtilis* (EPC016), a significant increase in seedling growth was observed relative to un-inoculated plants (Ramyabharathi et al., 2013). In another study, Tilak and Reddy (2006) observed a significant increase in grain yield rate of 43.8% in maize plants inoculated with *Bacillus cereus*. This last strain was found to exhibit the highest nitrogenase activity among 42 different strains of Bacillus spp studied by Ambrosini et al., (2016). In addition, *B. cereus* and *B. megaterium* have been reported as organic phosphorus mineralizing bacteria (Guang Can et al., 2008). The works of Habib et al., (2015, 2016) on rhizobacteria isolated from saline soil and selected for their PGP activities revealed that they showed significant salt tolerance properties. These rhizobacteria were identified as Enterobacter sp. and *Bacillus cereus*.

Our research indicates that significantly lower values of different growth parameters were recorded in maize plants inoculated with *Enterobacter ludwigii* in comparison to *Bacillus subtilis* and *Bacillus cereus*. On the other hand, and compared to the un-inoculated control plants, *Enterobacter ludwigii* had significant positive effects on maize plant growth parameters. Zaballa et al., (2020) found that barley plants inoculated with the *Enterobacter ludwigii* strain showed improvement in growth and phosphate uptake compared to the un-inoculated control. Tahir et al., (2013) reported that inoculation of wheat plants with phosphate-solubilizing and phytohormone-producing bacterial strains such as Azospirillum, Bacillus and Enterobacter improved growth and yield. Moreover, several studies have demonstrated the effectiveness of inoculating wheat grains with different rhizobacteria on plant growth (Abbasi et al., 2011; Rana et al., 2011; Banerjee et al., 2010). Numerous studies have highlighted the increase in dry matter weight of aerial parts in wheat (Bashan, 1986) and maize (García de Salamone and Döbereiner, 1996; Ullah et al., 2014; Di Salvo et al., 2018). The positive effects of PGPR on the yield and growth of crops such as wheat (Ozturk et al., 2003; Salanture et al., 2006) maize (Egamberdiyeva, 2007; Ullah S and B Asghari, 2015; Pereira et al., 2020) soybean (Cattelan et al., 1999) and sugar beet (Cakmakc et al., 2006) have been explained by the ability of these PGPR to fix N2, solubilize phosphate and produce phytohormones. Thus, these rhizobacteria can be considered as an excellent tool for increasing the availability of phosphorus in plants by mineralization of soil organic phosphorus and by solubilization of phosphate precipitates (Kucey et al., 1989; Pradhan and Sukla, 2006), production of AIA (Chaiharn and Lumyong, 2011; Swain et al., 2007), HCN (Bakker and Schippers, 1987), ammonia (NH3) (Yadav et al., 2010) and siderophores (Boopathi and Rao, 1999).

In general, our study clearly showed that the inoculation of maize plants with the rhizobacterial strains significantly promoted maize plants growth. These results suggest that these PGPR strains can be applied as biofertilizers for improving plants production. Furthermore, their use can be an ecological alternative to reduce the dependence on chemical fertilizers.

Conclusion

The rhizobacterial strains investigated in our study showed their plant growth ability. These native strains, which belong to the genera Bacillus and Enterobacter, significantly enhanced the growth of maize plants when compared with the un-inoculated control plants.

The maximal lengths of the maize seedlings were obtained with strain (I2: *Bacillus cereus*) (95.41 cm) with an increase of 33.45 % compared to un-inoculated (controls). The three isolates I2: *Bacillus cereus*, AZS2: *Bacillus subtilis* and commercial strain AZB: *Azospirillum brasilense*) caused a highly significant increase in the total number of leaves ranging from 10.9% to 12.7% compared to the un-inoculated control. Seedlings inoculated with AZS2: *Bacillus subtilis* strain exhibited the highest aerial dry biomasses with an improvement of more than 85 % (30.76 g) compared with un-inoculated control plants and more than 62 % compared to un-inoculated NaCl control plants. The inoculation treatment with I2: *Bacillus cereus* strain induced an improvement of more than 65 % (27.44g) over un-inoculated control and more than 45 % over un-inoculated NaCl control. The strain AZS2: *Bacillus subtilis* produced the highest root dry weights, in comparison to other isolates and induced an improvement of 30.17% (26.06 g) compared to un-inoculated plants (control) and 24.09% compared to un-inoculated plants (NaCl control). The most effective rhizobacterial treatment in the dry biomasses of whole seedling (aerial dry biomass and root dry biomass) is AZS2: *Bacillus subtilis* strain which induced an improvement of 55% (56.83 g) compared to un-inoculated plants (control) and 42% compared to un-inoculated plants (NaCl control). The most important production of kernels was recorded with AZS2: *Bacillus subtilis* strain.

Consequently, this finding suggests that these PGPR strains could be useful for the development of inoculants biofertilizers to improve the quality and the health of the soil and the plant species by increasing the nutrient availability for the soil and plants especially in arid and semi-arid regions. Furthermore, using biofertilizers that contain these rhizobacterial strains will led to a decrease in the use of chemical fertilizers and will provide high quality products free of harmful agrochemicals for human and environment.

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