Bacillus subtilis ameliorates water stress tolerance in maize and common bean

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ABSTRACT

Water stress is one important abiotic stress with negative impacts on plant productivity. In order to ameliorate abiotic stress, plant growth-promoting rhizobacteria (PGPR), such as Bacillus subtilis, can be used due to their positive effects on plant physiology. The present study aimed to evaluate the effects of B. subtilis on the performance of maize and common bean under water deficit conditions. The study was performed in a plant growth chamber and the growth, gas exchange parameters and antioxidant activity were evaluated. B. subtilis promoted the growth of common bean and maize, and also increased the water use efficiency. The inoculation with B. subtilis increased leaf water content and the regulation of stomata, without damaging photosynthetic rates. Overall, B. subtilis decreased antioxidant activities in both plants. The results suggest that B. subtilis could be used as inoculants for common bean and maize to protect against water stress.

1. Introduction

Water stress is one important abiotic stress that induces alterations in plant metabolism and development with negative impacts on plant productivity (Shirinbayan et al. 2019). Among the main crops, common bean and maize are quite sensitive to water stress. Usually, common bean production occurs under conditions where the risk of water stress is high (Beebe et al. 2013), while maize may decrease its yield due to water stress, partly because maize is originated from the wetter region, which probably contributed to its sensitivity under unfavorable climatic conditions (Daryanto et al. 2016). According to FAO (2018), water stress can lead to an estimated loss of a billion dollars to agriculture due to negative impacts on crop yield. In this view, the development of strategies to alleviate water stress has been required and the use of inoculants containing beneficial microorganisms deserves attention (Ngumbi and Kloeppep 2016).

Some bacteria belonging to the genera Azotobacter, Bacillus, Klebsiella, Pseudomonas, Azospirillum, and Serratia are beneficial microorganisms recognized as plant growth-promoting rhizobacteria (PGPR) (Figueiredo et al. 2016; Parray et al. 2016). PGPR are microbes that promote beneficial effects on plant development through direct or indirect mechanisms (Ngumbi and Kloeppep 2016; Enebe and Babalola 2018). Nutrient acquisition, synthesis of phytohormones, production of siderophore and improvement of antioxidant system are direct mechanisms performed by PGPR (Parray et al. 2016). Indirectly, PGPR can stimulate plant growth by triggering the plant immune system against phytopathogens (Lastochkina et al. 2017; Numan et al. 2018). In addition, PGPR play important roles on plant physiology, including the amelioration of abiotic stress responses (Figueiredo et al. 2008; Islam et al. 2015).

Among the most well-known PGPR, Bacillus subtilis has received particular attention because of its catabolic versatility and root colonization ability, as well as its capability to produce a large number of enzymes and metabolites that can favor plant growth under biotic and abiotic stress conditions (Gagné-Bourque et al. 2016). Indeed, studies have found B. subtilis increasing the photosynthetic capacity of plants through a direct influence on stomatal conductance and cell tolerance to dehydration (Cohen et al. 2008; Zhang et al. 2008; Li et al. 2016). In wheat, inoculation with B. subtilis induced higher rate of CO2 assimilation in plants exposed to water stress when compared to non-inoculated and stressed plants (Barnawal et al. 2017). Gagné-Bourque et al. (2016) observed positive effects on the photosynthetic rate in Timothy plants inoculated with B. subtilis and exposed to water stress. Overall, B. subtilis positively influences photosynthetic activity, being a promoter of water use efficiency in plants (Li et al. 2016).

Optimization of the water use efficiency, i.e. increasing the amount of carbon input by plants per unit of water, has been pointed out as a strategy to reduce the negative effects of water stress. According to Blum (2005), the increase in water use efficiency by the plants can be performed by the control of gases exchanged through stomata. However, it has been observed that the increase of water use efficiency by the reduction of stomatal conductance has decreased the photosynthetic rates and, consequently, crop performance (Fang and Xiong 2015). Some studies have reported the use of PGPR as promoters of water use efficiency in important crops, such as wheat, maize, and lettuce (Creus et al. 2004; Figueiredo et al. 2008; Marulanda et al. 2009). Li et al. (2016) observed that B. subtilis influenced the closure of stomata and promoted water use efficiency in Vicia faba.

PGPR can also mitigate negative effects of water stress by reducing oxidative damage caused by reactive oxygen species.
(ROS) through antioxidant enzymatic or ROS-scavenging activity, both regulated by the plants as a response to water stress (Kang et al. 2014; You and Chan 2015). Oxidative damage is due to the imbalance between production and scavenging of ROS and it is responsible for lipids damage and the collapse of chlorophyll (Meher et al. 2018). The ROS-scavenging activity of certain antioxidant compounds can be observed through the reaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) – a stable free radical widely used to study the ability to remove ROS (Nimse and Pal 2016). Kang et al. (2014) reported that the inoculation of Pseudomonas putida altered the antioxidant status of soybean under water stress and reduced the antioxidant activity in comparison to the control. The hypothesis of this study was that B. subtilis could ameliorate the effects of water stress on common bean and maize through the reduction of antioxidant activity. Thus, the aim of this study was to evaluate the effects of B. subtilis on the performance of common bean and maize under water stress conditions.

2. Materials and methods

2.1. Microorganism and inoculant production

Two strains of B. subtilis (AP-3 and PRBS-1) were used in this study. These strains were isolated from soil under soybean cultivation and characterized as PGPR by Araújo et al. (2005). The strains were maintained in nutrient-agar culture medium under controlled conditions. Aqueous suspensions containing each isolate at the concentration of 10⁹ cells mL⁻¹ were used as a bacterial inoculant.

2.2. Plant material and growth conditions

The experiment was carried out in a completely randomized design with three treatments: (a) plants non-inoculated; (b) plants inoculated with B. subtilis AP-3 or B. subtilis PRBS-1; (c) plants non-inoculated and inoculated under two water conditions, i.e. replacement of 30% and 100% of the evapotranspirated water. Maize (Zea mays L.; Hybrid SYN 7205) and common bean (Phaseolus vulgaris L.; cv. 'Estilo') seeds were sown in plastic pots containing 5.0 kg of soil and then inoculated with an aqueous suspension containing B. subtilis AP-3 or B. subtilis PRBS-1 (10⁵ cells mL⁻¹). Bacterial aqueous suspension was applied directly onto the seeds, in the planting, in the amount of 0.1 mL. The soil used in the experiment, classified as Acrisol (FAO 1998), was collected at 0–20 cm depth and present the following chemical properties: pH (CaCl₂), 5.9; P (Mehlich-1), 43.9 mg dm⁻³; K, 2.7 mmol dm⁻³; Ca, 25.3 mmol dm⁻³; Mg, 5.3 mmol dm⁻³.

After sowing, non-inoculated and inoculated common bean and maize plants were transferred to a plant growth chamber (Fitotron® SGC 120; Weiss Technik, UK) at 28/18°C (day/night) with a photoperiod of 16 h. Light intensity and relative humidity were kept at 300 μmol m⁻² s⁻¹ and 60%, respectively. Initially, soil moisture was kept at 100% of field capacity. After 15 days of plant emergence, different water conditions were induced and pots were kept at 100% (control) or 30% of the evapotranspired water (water stress). The daily control of evapotranspiration was carried out using the gravimetric method (Catuchi et al. 2011). Soil moisture contents were monitored by humidity and temperature sensors (Prochek®; Decagon Devices, Pullman, WA, USA).

2.3. Gas exchange measurements

At 35 days of plant emergence, the gas exchange parameters were measured by using a portable infrared gas analyzer system (Li-6400XTRA, LI-COR Biosciences, Lincoln, NE, USA). Gas exchange measurements were performed on five plants per treatment under photosynthetic photon flux density of 1200 μmol m⁻² s⁻¹. The following gas exchange parameters were evaluated: net CO₂ assimilation rate (mmol m⁻² s⁻¹), transpiration rate (mmol m⁻² s⁻¹), stomatal conductance (mmol m⁻² s⁻¹), and intercellular CO₂ concentration (ppm). Instantaneous carboxylation efficiency (mmol m⁻² s⁻¹ Pa⁻¹) was calculated as the ratio between net CO₂ assimilation rate and intercellular CO₂ concentration (in Pa), whereas water use efficiency (mmol mol⁻¹) was calculated as the ratio between net CO₂ assimilation rate and transpiration (Ribeiro et al. 2009).

2.4. Relative water content and relative chlorophyll content

Relative water content (RWC) of leaves was determined according to the method proposed by Barrs and Weatherley (1962) by using the fresh (FW), turgid (TW) and dry (DW) weight of leaf discs. The RWC was calculated through the formula: RWC (%) = (FW-DW)/(TW-DW) × 100 and expressed as percentage (%). The foliar chlorophyll content was obtained through a portable chlorophyll meter (CFL1030; Falkor Agricultural Automation, Porto Alegre, Brazil).

2.5. Plant growth analysis

At the harvest (40 days after plant emergence), the plants were collected, and roots and shoot were separated. The shoot was separated from the roots at 2.0 cm above the soil level and washed with water containing mild detergent to remove the soil. The quantification of roots and shoot dry weights were performed in the laboratory after drying the material in a forced ventilation oven (60–70°C). The evaluation of growth promotion was carried out by comparing the root and shoot dry weights of the inoculated plants and the control, within each water replacement condition.

2.4. Antioxidant activity

At 45 days after plant emergence, 50 g of dried leaves (at 30°C) of maize and common bean were subjected to extraction with 1.5 L of ethanol at room temperature and protected from light. The process of maceration and filtration of the supernatant was carried out in three consecutive stages, and each extraction lasted 30 min. The extracts obtained were combined and concentrated by vacuum evaporation. Extracts were then dried at 30°C in a forced air circulation oven. The antioxidant activity was assessed via free radical scavenging activity of the synthetic-free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The amount of antioxidants present in the ethanolic extracts required to decrease the initial concentration of DPPH by 50%, termed ‘inhibitory concentration (IC₅₀)’ was subsequently determined (Brand-Williams et al. 1995).

2.6. Data analysis

The data obtained as mean values of five replicates and significance were considered at the 95% confidence level.
Normality test and ANOVA was performed using SISVAR software (Ferreira 2014) to evaluate the effects of inoculation with B. subtilis on water stressed and non-stressed conditions. Tukey’s test was used to compare the means of each treatment.

3. Results

3.1. Effect of B. subtilis and water stress on plant growth and water status

The inoculation with B. subtilis and water stress displayed a significant effect (F-test; \( p < .05 \)) on shoot and root dry weight in common bean and maize (Table 1). Under non-stressful conditions (control), the inoculation of common bean with B. subtilis AP-3 resulted in an increase of 83% and 90% in shoot and root dry weight, respectively, as compared to non-inoculated plants (Table 2). However, common bean inoculated with B. subtilis PRBS-1, under non-stressful conditions, did not show different shoot dry weight as compared to non-inoculated plants (Table 2), while that the root dry weight increased by 31% from non-inoculated to inoculated plants. The shoot and root dry weight of maize inoculated with B. subtilis AP-3 under non-stressful conditions (control) were statistically similar to non-inoculated plants. In maize inoculated with B. subtilis PRBS-1, under non-stressful conditions, the shoot dry weight was increased by 10% when compared to non-inoculated plants and the root dry weight did not differ statistically from non-inoculated plants (Table 2).

The water stress induced reduction in shoot and root dry weight of inoculated and non-inoculated common bean and maize in relation to the non-stressful conditions, except to root dry weight in maize inoculated with B. subtilis PRBS-1 (Table 2). Under water stress, the inoculation of common bean with B. subtilis AP-3 or PRBS-1 did not induce a significant difference in shoot and root dry weight when compared to non-inoculated plants. The shoot dry weight of maize inoculated with B. subtilis AP-3 under water stress were statistically similar to non-inoculated plants (Table 2), whereas the inoculation with B. subtilis AP-3 had a significant effect on root dry weight and promoted an increase of 15% in root dry weight in relation to non-inoculated plants. Maize inoculated with B. subtilis PRBS-1 and exposed to water stress displayed significant increment in shoot and root dry weight (Table 2). These plants showed a 133% increase in shoot dry weight under water stress in relation to non-inoculated plants, while that root dry weight under water stress was increased by 103% when compared to non-inoculated plants.

3.2. Relative water content and chlorophyll index versus B. subtilis and water stress

Inoculation with B. subtilis (AP-3 or PRBS-1) promoted significant differences (F-test; \( p < .05 \)) in relative water content of common bean and maize, whereas water stress did not significantly influence this variable (Table 1). Under non-stressful conditions (control), common bean and maize inoculated with B. subtilis AP-3 or B. subtilis PRBS-1 showed the similar relative water content than non-inoculated plants (Figure 1). Under water stress, relative water content in common bean inoculated with B. subtilis AP-3 or B. subtilis PRBS-1 was increased in relation non-inoculated plants (Figure 1(A)). As showed in Figure 1(B), maize inoculated with B. subtilis AP-3 and exposed to water stress displayed 20% higher relative water content than non-inoculated and stressed plants.

The water stress presented a significant effect on chlorophyll content (Table 1). Under non-stressful conditions (control), common bean inoculated with B. subtilis PRBS-1 presented higher chlorophyll content (47.6 of Falker index). However, this result was statistically similar to the non-inoculated plants and also inoculated plants with B. subtilis AP-3 (Figure 2(A)). Water stress influenced negatively the chlorophyll content in non-inoculated common bean and those inoculated with B. subtilis PRBS-1 (Figure 2(A)), however common bean inoculated with B. subtilis AP-3 exhibited similar chlorophyll content than non-stressed plants. In maize, chlorophyll content did not differ in response to inoculation with B. subtilis in both control (non-stressful) and water stress conditions and when these conditions were compared (Figure 2(B)).

3.3. Effect of B. subtilis and water stress on gas exchange parameters

There was a significant effect of inoculation with B. subtilis on gas exchange parameters, except for net CO₂ assimilation rate (Table 1). Net CO₂ assimilation rate was statistically similar in plants inoculated with B. subtilis (AP-3 or PRBS-1) under control and water stress when compared to non-inoculated plants, in both common bean and maize (Figures 3 and 4). The transpiration rate was significantly reduced in common bean inoculated with B. subtilis AP-3 under non-stressful (control) and water stress conditions (Figure 3(B)). Maize inoculated with B. subtilis PRBS-1 showed reductions in transpiration rate under non-stressful (control) conditions when compared to non-inoculated plants. Under water stress, the

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**Table 1.** Effect of inoculation (I) or water stress (WS) and their combination (I × WS) in all variables analyzed on common bean and maize plants.

| Variables analyzed | Common bean | maize |
|--------------------|-------------|-------|
|                    | IN | WS | IN x WS | IN | WS | IN x WS |
| Shoot dry weight   | *  | *  | ns       | *  | *  | ns       |
| Root dry weight    | *  | *  | ns       | *  | *  | ns       |
| Relative water content | ns | *  | *  | ns | *  | *  |
| Chlorophyll index  | ns | ns | *  | *  | ns | *  |
| Net CO₂ assimilation rate | ns | ns | ns | ns | ns | ns |
| Transpiration rate | *  | *  | ns       | *  | *  | ns       |
| Stomatal conductance | *  | *  | *  | ns | *  | *  |
| Water use efficiency | *  | *  | *  | ns | *  | *  |
| ICE               | *  | *  | ns       | ns | ns | ns       |
| Antioxidant activity (DPPH) | *  | *  | *  | ns | *  | *  |

Notes: *Significant difference \((p < .05)\); ns = non-significant difference. *Instantaneous carboxylation efficiency.

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**Table 2.** Dry weight of shoot and root in common bean and maize submitted to water stress in the presence or absence of B. subtilis (AP-3 or PRBS-1).

| Treatments | Common bean | maize |
|------------|-------------|-------|
| Shoot dry weight (g plant⁻¹) | Control | Water stress | Control | Water stress |
| Non-inoculated | 0.76 aB | 0.44 aB | 0.80 aB | 0.52 aB |
| B. subtilis AP-3 | 1.39 aA | 0.56 aA | 1.52 aA | 0.49 aA |
| B. subtilis PRBS-1 | 0.83 aB | 0.36 aB | 1.05 aA | 0.53 aB |
| Maize | 1.90 aB | 0.69 bB | 0.83 aA | 0.39 bB |
| B. subtilis AP-3 | 1.84 aB | 0.79 bB | 0.80 aA | 0.45 bB |
| B. subtilis PRBS-1 | 2.07 aA | 1.61 aB | 0.88 aA | 0.79 aA |

Note: Means followed by distinct letters, lowercase in the columns and uppercase in the lines, differ by Tukey’s test at 5%.
The transpiration rate was 40% lower in maize inoculated with *B. subtilis* AP-3 or PRBS-1 in relation to non-inoculated plants (Figure 4(B)).

The stomatal conductance was significantly influenced by inoculation in both common bean and maize (Table 1) and the reduction in response to water stress was found only in common bean (Figure 3(C)). Under non-stressful conditions (control), stomatal conductance in common bean and maize inoculated with *B. subtilis* were reduced in 25% and 28%, respectively, when compared to non-inoculated plants (Figures 3(C) and 4(C)). When exposed to water stress, common bean inoculated with *B. subtilis* AP-3 presented a decrease in stomatal conductance as compared to non-inoculated plants (Figure 3(C)). Maize inoculated with *B. subtilis* AP-3 or with *B. subtilis* PRBS-1 and submitted to water stress showed reduction of 22% and 30% on stomatal conductance, respectively, compared to non-inoculated plants (Figure 4(C)).

Intracellular CO₂ concentration was not altered in the common bean (Figure 3(D)) and maize (Figure 4(D)) in response to the inoculation under non-stressful condition (control), whereas, under water stress, alterations in intracellular CO₂ concentration was recorded only in non-inoculated maize (Figure 4(D)). Maize inoculated with *B. subtilis* AP-3 or PRBS-1 and submitted to water stress exhibited an increase of 26% and 51% in intracellular CO₂ concentration, respectively, when compared to non-inoculated maize (Figure 4(D)). The instantaneous carboxylation efficiency was not altered in the common bean (Figure 3(E)), however, in maize inoculated with *B. subtilis*, there was an increase in this variable in relation to non-inoculated plants (Figure 4(E)). Overall, maize plants inoculated with *B. subtilis* showed an increase about 68% and 46% in the efficiency in relation to non-inoculated plants under control (non-stressful condition) and water stress, respectively (Figure 4(E)).

Both strains of *B. subtilis* improved the water use efficiency in common bean and maize under non-stressful (control) and water stress conditions (Figures 3(F) and 4(F)). Under non-stressful condition (control), common bean inoculated with *B. subtilis* AP-3 showed 44% increase in water use efficiency in relation to non-inoculated plants, while maize inoculated with *B. subtilis* AP-3 or with *B. subtilis* PRBS-1 exhibited water use efficiency 70% and 31% superior to non-inoculated plants, respectively (Figure 4(F)). Common bean exposed to water stress improved the water use efficiency in 11% and 19% when inoculated with *B. subtilis* AP-3 or PRBS-1, respectively. Similarly, water use efficiency in maize plants inoculated with *B. subtilis* AP-3 or PRBS-1 were 25% and 22% superior to non-inoculated plants, respectively, under water stress conditions (Figure 4(F)).

### 3.3. Antioxidant activities in response to *B. subtilis* and water stress

Common bean and maize showed different antioxidant activities under non-stressful (control) and water stress conditions (Figure 5). While common bean inoculated with *B. subtilis* AP-3 showed antioxidant activity similar to non-inoculated plants in non-stressful condition (Figure 5(A)). There was a reduction in antioxidant activity in common bean with *B. subtilis* PRBS-1, when compared to non-inoculated plants. However, maize under non-stressful condition showed an increased antioxidant activity when inoculated with *B. subtilis* (AP-3 or PRBS-1) in relation to non-inoculated plants (Figure 5(B)). The water stress influenced...
negatively the antioxidant activity in common bean (reduced by about 50%), except to common bean with B. subtilis PRBS-1 (Figure 5(A)). In contrast, maize inoculated with B. subtilis AP-3 exhibited an increase of 66% in antioxidant activity when compared to non-inoculated plants, both under water stress. Antioxidant activity in maize inoculated with B. subtilis PRBS-1 under water stress was 34% reduced in relation to non-inoculated plants (Figure 5(B)).

4. Discussion

Plant growth-promoting rhizobacteria (PGPR) include rhizosphere bacteria that can improve plant growth under different abiotic stress conditions (Numan et al. 2018). In this study, B. subtilis AP-3 and PRBS-1 were evaluated as PGPR on common bean and maize plants submitted to water stress conditions. Previous studies have reported these strains as potential PGPR (Araújo et al. 2005; Araújo 2008); however, their potential as plant growth promoters under water stress remain unclear. Our results showed that B. subtilis, especially the strain PRBS-1, was able to promote the shoot and root growth of maize plants and root growth of common bean plants (Table 2). Similar responses in wheat plants inoculated with B. subtilis and exposed to water stress were reported by Barnawal et al. (2017) and these authors suggest that this response is related of phytohormones released by the bacteria. As previously mentioned, PGPR release phytohormones directly in plant cells or in the rhizosphere and stimulate the plant growth (Numan et al. 2018). According to Lastochkina et al. (2017), B. subtilis promotes plant growth and increases plant stress tolerance through of direct and indirect ways, such as synthesis of siderophores and/or plant hormones and improvement of nutrient availability. Furthermore, it is related that plants associated with PGPR display better relative water content, especially in abiotic stress situations (Parray et al. 2016; Enebe and Babalola 2018). Indeed, common bean and maize inoculated with B. subtilis showed highest relative water content than non-inoculated plants under water stress. Relative water content reflects the leaf water status (Flexas et al. 2013; Numan et al. 2018) and the enhancement in this variable, due to inoculation with B. subtilis, can represent an important strategy to tolerate water stress (Barnawal et al. 2017).

Under water stress, stomatal conductance and transpiration are primarily reduced to restrict the water losses. Although the results have shown that the inoculation with B. subtilis did not alter photosynthesis, both strains of B. subtilis promoted a decrease in the stomatal conductance and transpiration rate of common bean and maize submitted to water stress. Similar responses were reported in pepper plants inoculated with Bacillus sp. (Samaniego-Gámez et al. 2016), in V. faba plants inoculated with B. subtilis (Li et al. 2016), and in tomato plants inoculated with Pseudomonas chlororaphis (Brilli et al. 2019). The negative effect of B. subtilis on

Figure 3. Gas exchange parameters in common bean plants submitted to water stress in presence or absence of Bacillus subtilis (AP-3 or PRBS-1): Net CO₂ assimilation rate (A), transpiration rate (B), stomatal conductance (C), intercellular CO₂ concentration (D), instantaneous carboxylation efficiency (E), and water use efficiency (F). In control or water stress, different lowercase letters represent significant differences among non-inoculated (NI) plants and those inoculated with B. subtilis (AP-3 or PRBS-1). Double asterisk (**) indicates significant differences between control and water stress in each treatment (Tukey’s test, p < 0.05).
stomatal conductance can be correlated with the production and liberation of abscisic acid by this bacterium (Araújo et al. 2005) since as this phytohormone is considered a potent regulator of stomatal conductance. According to Brilli et al. (2019) the improvement of water use efficiency is necessary to maintain low stomatal conductance followed by an inferior transpiration rate. The decrease in the transpiration rates and stomatal conductance of maize and common bean correlated with an increase in the relative water content of leaves and, consequently, the efficiency of water use. Interestingly, it was found a positive correlation between stomatal conductance and transpiration rate indicating that plants inoculated with both strains of *B. subtilis* (AP-3 and PRBS-1) were more efficient in improving water use efficiency under water stress condition and therefore have the potential to ameliorate the negative effects of water stress on important physiological traits. These results are in agreement with previous studies that reported *B. subtilis* has a potential to ameliorate water stress in plants (Vardharajula et al. 2010; Del Amor and Cuadra-Crespo 2012; Ahmad et al. 2013; Stefan et al. 2013; Li et al. 2016).

Under water stress, ROS are significantly accumulated and might cause oxidative damage to macromolecules and others cell compounds resulting in cellular damage and cell death
(You and Chan 2015; Ngumbi and Kloeper 2016; Enebe and Babalola 2018). According to Brilli et al. (2019), PGPR might improve plant performance in abiotic stress situations through maintenance of a ROS level compatible with cellular functioning. In our study, the antioxidant activity was reduced under water stress in non-inoculated or inoculated common bean (B. subtilis AP-3) and maize plants inoculated with B. subtilis PRBS-1. Similarly, the inoculation with P. putida decreased the antioxidant activity, measured by DPPH, in non-stressed maize plants (Kang et al. 2014). Conversely, maize plants treated with B. subtilis showed reduced antioxidant activity both under control conditions and under water deficit.

The results of this study showed that the inoculation of B. subtilis in common bean and maize plants can influence important photosynthetic traits in plants under water stress. Furthermore, the responses of each plant species to inoculation were different. The results showed that the reduction of leaf transpiration was the most consistent parameter influenced by B. subtilis against the water stress conditions. Nevertheless, as common bean and maize are important crops in tropical regions, and are affected by water stress conditions. This study suggests that plants inoculated of B. subtilis could improve their growth under water stress conditions, mainly due to increased water use efficiency that consequently maintains adequate leaf water status and plant biomass accumulation in these adverse conditions.

5. Conclusions
The strains AP-3 and PRBS-1 of B. subtilis increased the water use efficiency in common bean and maize plants by about 20%, without decreasing photosynthesis and plant growth. Specifically, B. subtilis PRBS-1 doubled the growth of the maize plants exposed to water stress. The main mechanisms involved in the increase of water use efficiency were the closure of stomata and the reduction of leaf transpiration, with an increase in leaf water content. Our results suggest that B. subtilis PRBS-1 and AP-3 strains can be used as potential plant growth promoters for common bean and maize during water stress conditions.

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