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Corn Yield and Phosphorus Use Efficiency Response to Phosphorus Rates Associated With Plant Growth Promoting Bacteria

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The use of plant growth promoting bacteria (PGPB) that can solubilize phosphorus (P) has shown potential to improve nutrient availability in grass crops such as corn (*Zea mays* L.) This study was developed to investigate if inoculation with *Azospirillum brasilense*, *Bacillus subtilis* or *Pseudomonas fluorescens* associated with P_2O_5 rates can improve phosphorus use efficiency (PUE) reflecting on greater corn development and yield. The field trial was set up in a Rhodic Hapludox under no-till system under Savannah conditions, in a completely randomized block design with four replicates. Treatments were tested in a full factorial design and included: (i) five P_2O_5 rates (0 to 105 kg ha^{-1}) and (ii) four PGPB seed inoculation (Control–without inoculation, *A. brasilense*, *B. subtilis* or *P. fluorescens*). Inoculation was found to increase grain yield by 39.5, 29.1, and 15.9% when *B. subtilis* was inoculated in the absence of P_2O_5 rates and associated with 17.5 and 70 kg P_2O_5 ha^{-1} and by 34.7% when *A. brasilense* was inoculated with application of 105 kg P_2O_5 ha^{-1}. In addition, inoculation with *B. subtilis* and *A. brasilense* were found to increase P uptake, benefiting productive components development, leading to an improved PUE, and greater corn grain yield. The results of this study showed positive improvements in P uptake as a result of *B. subtilis* and *A. brasilense* inoculation, with an increase of 100.5 and 54.6% on PUE, respectively; while the *P. fluorescens* inoculation were less evident. Further research should be conducted under biotic or/and abiotic conditions such as attack of pathogens and insects, drought, salinity, water flooding, low and high temperature to better understand the role of PGPB, inoculated alone or in combination as the co-inoculated method.

Keywords: *Azospirillum brasilense*, *Bacillus subtilis*, phosphorus fertilization management, *Pseudomonas fluorescens*, *Zea mays* L.

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INTRODUCTION

Phosphorus (P) is one of the most limiting nutrients in agricultural cropping systems (Roberts and Johnston, 2015; Guignard et al., 2017; Khan et al., 2018). It is estimated that P deficiencies can be found in nearly 67% of world land designated for crop production (Dhillon et al., 2017). Also, P use efficiency (PUE) for cereal production in the world is too low, varying between 15 and 30% (Dhillon et al., 2017). Under tropical conditions, P can precipitate as minerals of Fe, and Al (Penn and Camberato, 2019). Both minerals decrease the availability of P for plant growth (Dhillon et al., 2017). Clay fractions such as amorphous hydrated oxides of Fe and Al, in addition to gibbsite, goethite, and kaolinite are responsible for the greatest P fixation (Dhillon et al., 2017). This has created a cascade of environmental problems (e.g., global warming, air pollution, and eutrophication) that threaten ecosystems and human health (Gu et al., 2015). Therefore, the P demand by crops must be considered. Plants of intense and short-cycle development, such as the corn plant (*Zea mays* L.), require higher amounts of P in solution and faster adsorbed-P replenishment than perennial crops (Lino et al., 2018). However, fertilizer application is one of the highest input costs for cereal crops and yet most of the P-fertilizer used to supplement crops is lost into the environment, due to the low PUE in cereal crops (Metson et al., 2016; Li et al., 2017).

The use of agroecological practices such as inoculation by plant growth-promoting bacteria (PGPB) can represent a sustainable alternative for increase nutrient use efficiency in tropical agriculture (Galindo et al., 2018a,b, 2019a,b; Martins et al., 2018). The use of these PGPB is growing, particularly in Latin America, for different crops (Souza et al., 2015; Martins et al., 2018; Galindo et al., 2019a). Several PGPB genera show association with different species of agricultural importance, such as *Azospirillum*, *Bacillus* and *Pseudomonas* (Zeffa et al., 2018). These bacteria can stimulate plant growth by a series of mechanisms, including but not restricted, the production of phytohormones, such as salicylic acid, gibberellins, cytokinins and indole-3-acetic acid (IAA) (Cassán and Diaz-Zorita, 2016; Fukami et al., 2017), phosphate solubilization (Ludueña et al., 2018; Qi et al., 2018), nutrient availability increase (Galindo et al., 2018b), production of indolic compounds and siderophores (Ambrosini and Passaglia, 2017), increase on 1−aminocyclopropane−1−carboxylate deaminase activity (Ambrosini and Passaglia, 2017), biological nitrogen fixation (BNF) (Pankievicz et al., 2015), biological control of plants, production of natural antibiotics and protective effect against secondary soil phytopathogens (Zhou et al., 2016; Mishra and Arora, 2018; Shameer and Prasad, 2018).

The *Azospirillum* spp. is considered one of the most studied plant growth promoter genera (Galindo et al., 2016, 2017). An analysis of field trials conducted worldwide for over 20 years, where various non-legume crops were inoculated with *Azospirillum* spp. under different weather and soil conditions, concluded that crop yield can increase up to 30% with inoculation (Fukami et al., 2016). Also, positive results in corn development and yield has been reported with *Azospirillum brasilense* inoculation (strains Ab-V5 and Ab-V6) under tropical conditions (Martins et al., 2018; Oliveira et al., 2018; Galindo et al., 2019b). However, greater responses with other PGPB can be achieved (Pankievicz et al., 2019). New research investigating *Bacillus* spp. and *Pseudomonas* spp. as beneficial PGPB are being conducted, especially for annual crops (Oliveira et al., 2019; Pankievicz et al., 2019; Tavanti et al., 2020). For example, under tropical conditions, *Bacillus subtilis* inoculation (strains Pant001 and QST713) associated with *Bradyrhizobium japonicum* has been reported to increase soybean [*Glycine max* (L.) Merr.] yield compared to single inoculation with *B. japonicum*, besides improving seed quality due to the increase in total storage proteins concentration, seedling emergence percentage and seed vigor (Tavanti et al., 2020). Traoré et al. (2016) reported improved corn seed germination, plant growth, plant production (increase yield by 42%) grain and shoot P biomass content of 34 and 64%, respectively with *B. subtilis* inoculation (strain DSM10). Lima et al. (2019) verified that *B. subtilis* (strains AP-3 and PRBS-1) promoted common bean (*Phaseolus vulgaris* L.) and corn growth, increasing the water use efficiency, leaf water content and the regulation of stomata, without damaging photosynthetic rates. Zarei et al. (2019) concluded that *Pseudomonas fluorescens* (P1, P3, P8, and P14 – prepared from the collection of Vali-e-Asr University of Rafsanjan) can improve plant water deficit stress tolerance, P solubilization and siderophore production, leading to an increased sweet corn (*Zea mays* L. var saccharata) growth and yield. Differently, Oliveira et al. (2019), studying six PGPB inoculation in soybean (*A. brasilense*, *B. amyloliquefacens*, *B. licheniformis*, *B. pumilus*, *B. subtilis* e *P. fluorescens*) associated with *B. japonicum* did not verified plant development and increased grain yield compared to single inoculation with *B. japonicum*.

Understanding the success or failure of inoculation requires understanding the complex interactions between the roots of inoculated plants, the specificity between hosts and PGPB, and the major microbial communities in the rhizosphere (Bashan et al., 2004; Florio et al., 2017, 2019). Therefore, studies with different PGPB inoculation such as *Bacillus* spp. and *Pseudomonas* spp. in tropical conditions should be performed, since new reports can be largely applicable to other important producing countries. Regardless of several benefits may be verified with PGPB inoculation, an increase in PUE and corn grain yield is not always the case (Bounaffaa et al., 2018). Further research with different PGPB inoculation associated with P_2O_5 rates are needed to determine how to maximize its benefits on PUE, corn development and yield. This study was based on the hypothesis of positive effect between the different PGPB inoculation and P utilization, providing greater nutrient use efficiency, reflecting on corn development and yield. The objective of this study was to evaluate the effect of *A. brasilense*, *B. subtilis*, and *P. fluorescens* inoculation and P_2O_5 application rates, on the nutritional, productive components PUE and corn grain yield under tropical conditions.

MATERIALS AND METHODS

The study was conducted under field conditions in Selvíria (Savannah region), state of Mato Grosso do Sul, Brazil [20°22′ south (S) and 51°22′ west (W), 335 m above sea level (a.s.l.)], during the crop year of 2016/17. The soil was classified as clayey Oxisol (Rhodic Hapludox) according to the Soil Survey Staff (2014). The experimental area was cultivated with annual crops (cereal and legume crops) for over 30 years, with the last 12 years using a no-tillage system. The last crop sequence prior to corn was wheat. The maximum and minimum temperatures, air relative

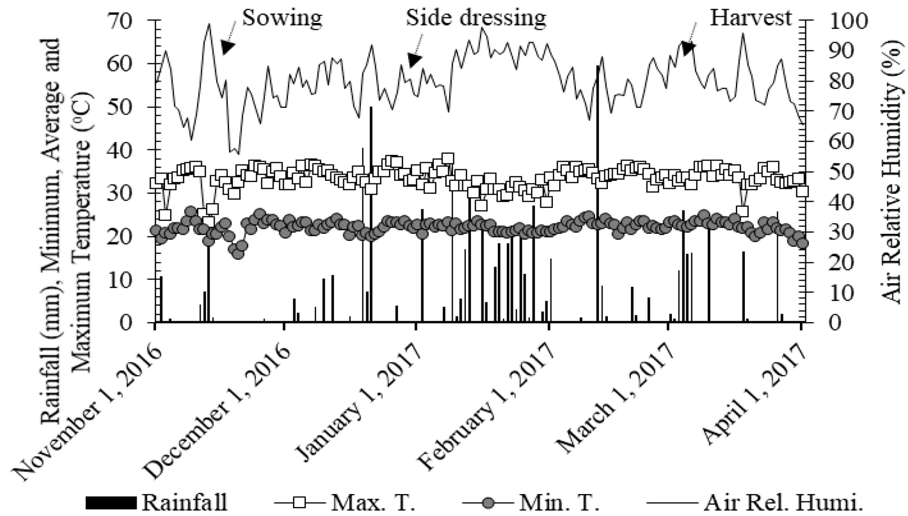

FIGURE 1 | Rainfall, air relative humidity, maximum and minimum temperatures obtained from the weather station located in the Education and Research Farm of FE/UNESP during the corn cultivation in the period November 2016 to April 2017.

humidity and the rainfall verified during the study are presented in **Figure 1**.

The experimental design was a completely randomized block design with four replicates arranged in a 4 × 5 factorial scheme: four PGBP seed inoculations (control–without inoculation, *A. brasilense*, *B. subtilis* and *P. fluorescens* and five P_2O_5 rates (0, 17.5, 35, 70, and 105 kg ha^{-1}) The experimental plots were composed of seven 3.5 m corn rows spaced at the distance of 0.45 m, with the useful area of the plot being the central three rows, with the exclusion of 0.5 m from each end.

Twenty soil samples were collected, mixed and a random sub-sample was used to determine soil chemical attributes before the beginning of field trial in the 0.0–0.2 m depth. This samples were collected with soil core sample type cup auger (0.4 m × 0.10 m–cup length and diameter, respectively), randomized in the entire experimental site, regardless of experimental blocks and plots. After samples be collected and mixed, the sub-sample was dried in the shade and soil chemical attributes were determined according to the Raij et al. (2001) methodology. The following results were verified in **Table 1**.

TABLE 1 | Soil chemical attributes in 0–0.20 m layer before field trial beginning.

Soil chemical attributes	0–0.20 m layer
P (resin)	20 mg dm^{-3}
S (SO_4)	3 mg dm^{-3}
Organic matter	24 g dm^{-3}
pH ($CaCl_2$)	5.3
K	1.6 $mmol_c$ dm^{-3}
Ca	33.0 $mmol_c$ dm^{-3}
Mg	20.0 $mmol_c$ dm^{-3}
H + Al	28.0 $mmol_c$ dm^{-3}
B (hot water)	0.19 mg dm^{-3}
Cu (DTPA)	3.9 mg dm^{-3}
Fe (DTPA)	21.0 mg dm^{-3}
Mn (DTPA)	63.5 mg dm^{-3}
Zn (DTPA)	1.6 mg dm^{-3}
Base saturation	68%

n = 20.

Weed control was performed with herbicides application of glyphosate [1800 g ha^{-1} of the active ingredient (a.i)] and 2,4-D (670 g ha^{-1} a.i). The mineral N and K fertilization was performed with 30 kg N ha^{-1} (urea) and 60 kg K_2O ha^{-1} (potassium chloride) at seedling and for all treatments, based on the soil analysis and corn crop requirements. Also, the application of P_2O_5 rates was performed at seedling based on soil analysis and crop requirements. The P_2O_5 rates (0, 17.5, 35, 70 e 105 kg ha^{-1}) corresponds to 0, 25, 50, 100, and 150% of the recommended rate, respectively (Cantarella et al., 1997). The source of P_2O_5 applied was triple superphosphate (18% of P_2O_5, 16% of Ca, and 8% of S).

The inoculation with *A. brasilense* strains Ab-V5 and Ab-V6 was performed at a dose of 100 mL of liquid inoculant per hectare [equivalent to 100 mL of inoculant per 73,400 corn seeds planted – guarantee of 2 × 10^8 CFU (colony forming unity) mL^{-1}]. The inoculation with *B. subtilis* strain CCTB04 was performed at a dose of 100 mL of liquid inoculant per hectare (equivalent to 100 mL of inoculant per 73,400 corn seeds planted – guarantee of 1 × 10^8 CFU mL^{-1}). The inoculation with *P. fluorescens* strain CCTB03 was performed at a dose of 100 mL of liquid inoculant per hectare (equivalent to 100 mL of inoculant per 73,400 corn seeds planted – guarantee of 1 × 10^8 CFU mL^{-1}). These are commercial strains used in Brazil [for both *A. brasilense* (brand name AzoTotal), *B. subtilis* (brand name Vult) and *P. fluorescens* (brand name Audax)]. Both inoculations (*A. brasilense*, *B. subtilis*, and *P. fluorescens*) were performed by coating and mixing the inoculants and corn seeds in plastic bags, manually. The seed inoculations were realized one hour before planting the corn crop and after seed treatment with fungicide and insecticide [the fungicides thiophanate-methyl + pyraclostrobin (56 g + 6 g of a.i. per 100 kg of seed) and the insecticide fipronil (62 g of a.i. per 100 kg of seed) were used], when the seeds were completely dry. The control treatment did not receive inoculations, however, the chemical seed treatment with fungicide and insecticide was performed, similarly to inoculated treatments.

The corn simple hybrid used was DOW 2B710 PW and the planting took place on November 11, being planted 73,400 seeds per ha. Seedling emergence occurred 5 days after sowing, on November 16, 2016. When necessary, the corn crop was irrigated with supplementary irrigation, using a center pivot sprinkling system (water depth of 14 mm). Weed and insect control were performed according to crop demand.

Nitrogen fertilizer (side dress application) was spread on the soil surface without incorporation by placing the fertilizer in the middle of the rows when the plants were in the V4 stage (with four leaves completely unfolded) at the dose of 150 kg N ha^{-1} as urea source, for all plots. After N-fertilization, the area was irrigated (14 mm depth) at night to minimize losses by ammonia volatilization. The plants were harvested manually at 125 days after emergence (DAE) on March 21, 2017.

The following nutritional evaluations were performed: (a) P foliar concentration, in g kg^{-1} of dry matter, was determined by collecting the middle third of 20 leaves of the main ear insertion in each experimental plot in the female flowering stage, according to the methodology described in Cantarella et al. (1997). Also, the (b) P concentration in biomass and grains were determined, at harvest time, and the P uptake in biomass and grains were calculated, in kg ha^{-1}. P determinations in tissue and grains followed the methodology that was proposed in Malavolta et al. (1997). Five soil samples (depth of 0–0.20 m) per plot were collected at the harvest time to determine (c) P-resin according to Raij et al. (2001).

The following productive components measurements were performed: (d) plant height at maturity, defined as being at a distance (m) from the ground level to the apex of the tassel; (e) stem diameter in the second internode at corn maturation plant using a manual caliper. Ten corn spikes were collected at the harvest time to follow the evaluations: (f) spike diameter; (g) spike length, determined from the base of the spike to the apex; (h) number of rows per spike, obtained by counting the number of all rows in each spike; (i) number of grains per row of spike, determined by counting the number of grains in each row of the spike; (j) number of grains per spike, obtained by counting the number of grains in each spike; (k) mass of 100 grains, determined at 13% moisture (wet basis) by a 0.01 g precision scale; (l) phosphorus use efficiency (PUE) following the Eq. 1; and (m) grain yield, determined by the spike collection in the useful experimental area. After the mechanical track, the grains were quantified and corrected to 13% moisture (wet basis), in kg ha^{-1}.

$$\text{PUE, adapted of Moll et al. (1982)}: \quad [(\text{GYF} - \text{GYW}) \div (\text{amount of N applied})] \tag{1}$$

Where, GYF = Grain Yield with fertilizer and GYW = Grain Yield without fertilizer.

The data was analyzed by ANOVA in a 2-way factorial design with P_2O_5 application rates and inoculation and their interactions considered fixed effects in the model. Mean separation was done when significant factors or interactions were observed using the test Tukey. Regression analysis was used to discern whether there was a linear or non-linear response to P_2O_5 rates in R software (R Core Team, 2015).

RESULTS

Statistical analysis showed that leaf P concentration, biomass P uptake, grain P uptake, plant height, ear length, ear diameter, number of grains per row, and grains per ear, mass of 100 grains, PUE and grain yield were significantly affected by the interaction between P_2O_5 rates × PGPB inoculation (**Table 2**). Phosphorus resin in soil and stem diameter were significantly affected by the main effects of P_2O_5 rates and PGPB inoculation (**Table 2**). Number of rows per ear was not affected by P_2O_5 rates, PGPB inoculation or the interaction between the factors (**Table 2**).

Control plots associated with low and average P_2O_5 application rates (0, 17.5 and 35 kg ha^{-1}) resulted in greater leaf P concentration compared to *A. brasilense*, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 2A**). Also, control plots

TABLE 2 | F-values for leaf P concentration, biomass P uptake, grain P uptake, P-resin in soil, plant height, stem diameter, ear length, ear diameter, number of rows per ear, grains per row and grains per ear, mass of 100 grains, phosphorus use efficiency and corn grain yield 2016/17.

F-values	Leaf P concentration	Biomass P uptake	Grain P uptake	P-resin in soil	Plant height	Stem diameter	Ear length
P_2O_5 rates (R)	5.691**	3.405*	13.019**	10.938**	3.052*	2.756*	2.164^{ns}
PGBP inoculation (I)	75.765**	0.828^{ns}	8.017**	11.606**	19.637**	23.992**	7.976**
R x I	3.968**	3.043**	3.073**	1.285^{ns}	3.685**	1.642^{ns}	3.112**
F-values	**Ear diameter**	**Rows per ear**	**Grains per row**	**Grains per ear**	**Mass of 100 grains**	**Phosphorus use efficiency**	**Grain yield**
P_2O_5 rates (R)	2.809*	1.462^{ns}	1.740^{ns}	2.168^{ns}	1.635^{ns}	26.856**	14.060**
PGBP inoculation (I)	2.124^{ns}	1.815^{ns}	2.510^{ns}	3.085*	1.795^{ns}	6.624**	11.287**
R x I	2.320*	1.514^{ns}	3.452**	1.957*	2.553**	2.946**	3.471**

***, * and ns: significant at $p < 0.01$, $p < 0.05$, and not significant, respectively.*

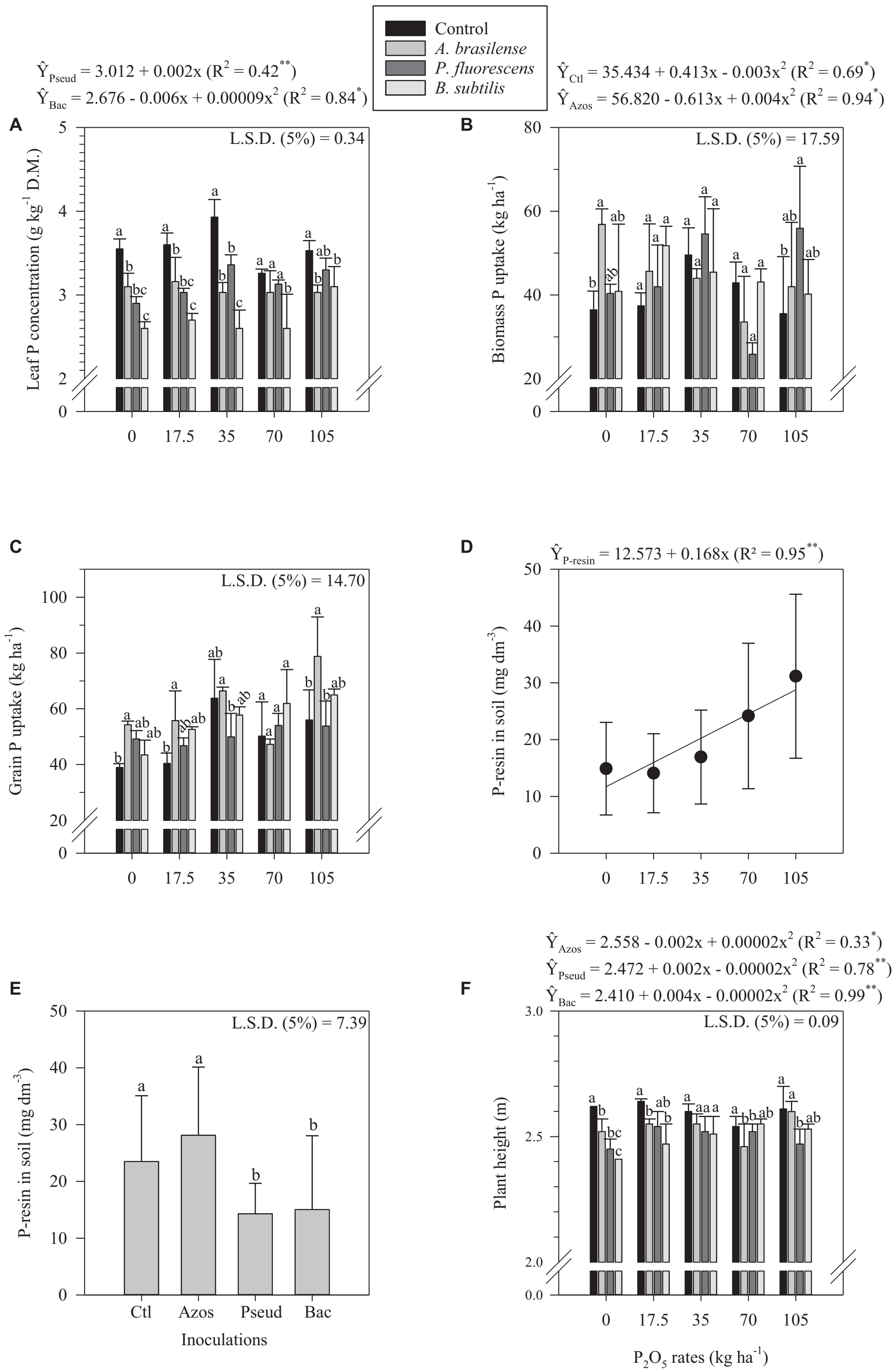

FIGURE 2 | Leaf P concentration **(A)**, Biomass P uptake **(B)**, Grain P uptake **(C)** as a function of the interaction between P_2O_5 rates and inoculations, P-resin in soil as a function of P_2O_5 rates **(D)** and inoculations **(E)** and plant height **(F)** as a function of the interaction between P_2O_5 rates and inoculations in corn crop. The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). Error bars indicate the standard deviation of the mean ($n = 4$). Ctl, Azos, Pseud e Bac refers to the control treatment and inoculations with *A. brasilense*, *P. fluorescens* and *B. subtilis*, respectively. L.S.D. (least significant difference), D.M. (dry matter).

associated with high P_2O_5 rates (105 kg ha^{-1}) resulted in greater leaf P concentration compared to *A. brasilense* and *B. subtilis* inoculated plots (**Figure 2A**). Leaf P concentration responded linearly to P_2O_5 application rates when *P. fluorescens* was inoculated (**Figure 2A**). Differently, leaf P concentration responded non-linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 2A**).

In the absence of P_2O_5 application, control plots resulted in lower biomass P uptake compared to *A. brasilense* inoculated plots (**Figure 2B**). Also, control plots associated with high P_2O_5 application rates (105 kg ha^{-1}) resulted in lower biomass P uptake compared to *P. fluorescens* inoculated plots (**Figure 2B**). Biomass P uptake responded non-linearly to P_2O_5 application rates without inoculation and when *A. brasilense* was inoculated (**Figure 2B**). The *A. brasilense* inoculated plots associated with low and high P_2O_5 application rates (0, 17.5 and 105 kg ha^{-1}) resulted in greater grain P uptake compared to control plots (**Figure 2C**). In addition, *A. brasilense* inoculated plots associated with 35 kg P_2O_5 ha^{-1} resulted in greater grain P uptake compared to *P. fluorescens* inoculated plots (**Figure 2C**). Grain P uptake responded linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 2C**). Differently, grain P uptake responded non-linearly to P_2O_5 application rates when control and *A. brasilense* was inoculated (**Figure 2C**). Phosphorus resin in soil responded linearly to P_2O_5 application rates (**Figure 2D**). In addition, control and *A. brasilense* inoculated plots resulted in greater P-resin in soil compared to *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 2E**).

Control plots associated with low P_2O_5 application rates (0 and 17.5 kg ha^{-1}) resulted in greater plant height compared to *A. brasilense*, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 2F**). In addition, control plots associated with 105 kg P_2O_5 ha^{-1} resulted in greater plant height compared to *P. fluorescens* inoculated plots (**Figure 2F**). However, *B. subtilis* inoculated plots associated with 70 kg P_2O_5 ha^{-1} resulted in greater plant height compared to *A. brasilense* inoculated plots (**Figure 2F**). Plant height responded non-linearly to P_2O_5 application rates when *A. brasilense*, *P. fluorescens* and *B. subtilis* were inoculated (**Figure 2F**). Stem diameter responded linearly to P_2O_5 application rates (**Figure 3A**). In addition, *A. brasilense* inoculated plots resulted in greater stem diameter compared to control, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 3B**).

Ear length fluctuated throughout the P_2O_5 application rates; however, in general, control plots showed reduced ear length compared to *A. brasilense*, *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 3C**). Ear length responded linearly to P_2O_5 application rates when *P. fluorescens* was inoculated (**Figure 3C**). Differently, ear length responded non-linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 3C**). Control plots and *P. fluorescens* inoculated plots associated with average P_2O_5 rates (35 and 70 kg ha^{-1}) tended to result in lower ear diameter compared to *A. brasilense* and *B. subtilis* inoculated plots (**Figure 3D**). Ear diameter responded non-linearly to P_2O_5 application rates when control and *P. fluorescens* was inoculated (**Figure 3D**).

In the absence of of P_2O_5 application, *B. subtilis* inoculated plots resulted in greater number of grains per row compared to control, *A. brasilense* and *P. fluorescens* inoculated plots (**Figure 3E**). Grains per row responded non-linearly to P_2O_5 application rates when control and *B. subtilis* was inoculated (**Figure 3E**). Similarly, in the absence of of P_2O_5 application, *B. subtilis* inoculated plots resulted in greater number of grains per ear compared to control, *A. brasilense* and *P. fluorescens* inoculated plots (**Figure 3F**). Also, *A. brasilense* inoculated plots associated with 105 kg P_2O_5 ha^{-1} resulted in greater number of grains per ear compared to control plots (**Figure 3F**). Control plots and *P. fluorescens* inoculated plots associated with average and high P_2O_5 application rates (70 and 105 kg ha^{-1}) tended to result in lower mass of 100 grains compared to *A. brasilense* and *B. subtilis* inoculated plots (**Figure 4A**). Mass of 100 grains responded linearly to P_2O_5 application rates when *B. subtilis* was inoculated (**Figure 4A**). Differently, mass of 100 grains responded non-linearly to P_2O_5 application rates when control and *A. brasilense* and *P. fluorescens* were inoculated (**Figure 4A**).

The *B. subtilis* inoculated plots associated with 17.5 kg P_2O_5 ha^{-1} resulted in greater PUE compared to control, *A. brasilense* and *P. fluorescens* inoculated plots (**Figure 4B**). Phosphorus use efficiency responded linearly to P_2O_5 application rates regardless of inoculations (**Figure 4B**). The *B. subtilis* inoculation also benefited grain yield when associated with low P_2O_5 rates (0 and 17.5 kg ha^{-1}) as verified by greater grain yield compared to control (**Figure 4C**). The *A. brasilense* inoculated plots associated with 70 kg P_2O_5 ha^{-1} resulted in lower grain yield compared to *P. fluorescens* and *B. subtilis* inoculated plots (**Figure 4C**). However, *A. brasilense* inoculated plots associated with 105 kg P_2O_5 ha^{-1} resulted in greater grain yield compared to control and *P. fluorescens* inoculated plots (**Figure 4C**). Grain yield responded linearly to P_2O_5 application rates when *A. brasilense* was inoculated (**Figure 4C**). Differently, grain yield responded non-linearly to P_2O_5 application rates when control and *P. fluorescens* were inoculated (**Figure 4C**).

DISCUSSION

Phosphorus is the second nutrient that is most demanded by corn plants and directly affects crop development and yield (Dhillon et al., 2017). Thus, the higher P availability as a function of the P_2O_5 application probably favored initial root system development, reflecting on corn grain yield. The better growth of P_2O_5-fertilized plants can be attributed to the P readily available for absorption after being added to the soil as verified by the linear increasing response in P content in soil to P_2O_5 application rates. Phosphorus plays important roles in plant nutrition and development (Lollato et al., 2019), for example composition of adenosine triphosphate (ATP). In addition, P is responsible for the storage and transport of energy for endergonic processes, such as the synthesis of organic compounds and the active uptake of nutrients (Marschner, 2012). Also, P is related to root system development and plant growth (Sulieman and Tran, 2015; Fink et al., 2016; Zhang et al., 2016). There are several studies reporting the P fertilization benefits in corn crop (Wen et al., 2016; Li et al., 2017; Schlegel and Havlin, 2017; Ortas and Islam, 2018; Preston et al., 2019). However, plant nutritional demand

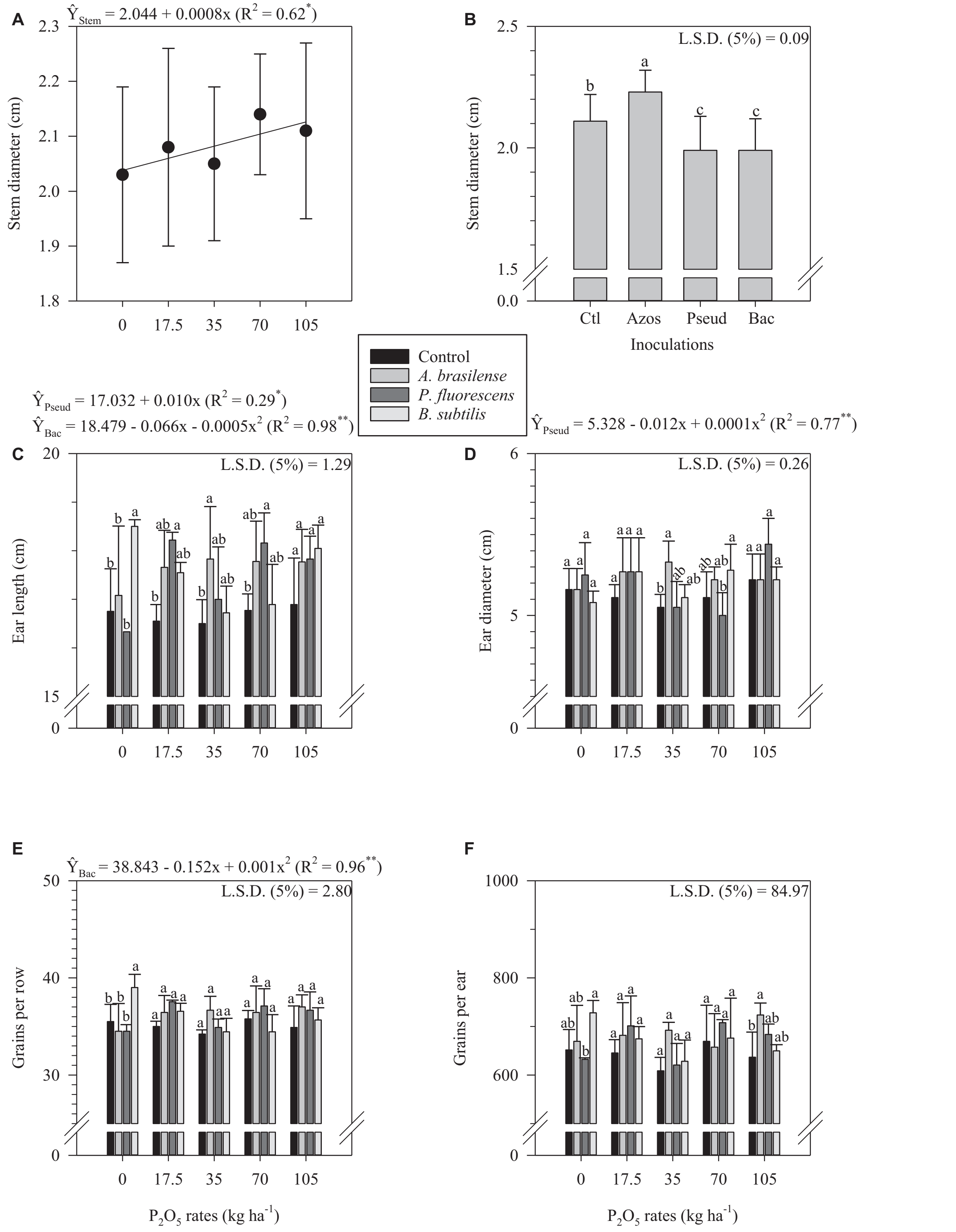

FIGURE 3 | Stem diameter as a function of P_2O_5 rates **(A)** and inoculations **(B)**, ear length **(C)**, ear diameter **(D)**, grains per row **(E)**, and grains per ear **(F)** as a function of the interaction between P_2O_5 rates and inoculations in corn crop. The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). Error bars indicate the standard deviation of the mean ($n = 4$). Ctl, Azos, Pseud e Bac refers to the control treatment and inoculations with *A. brasilense*, *P. fluorescens* and *B. subtilis*, respectively. L.S.D. (least significant difference).

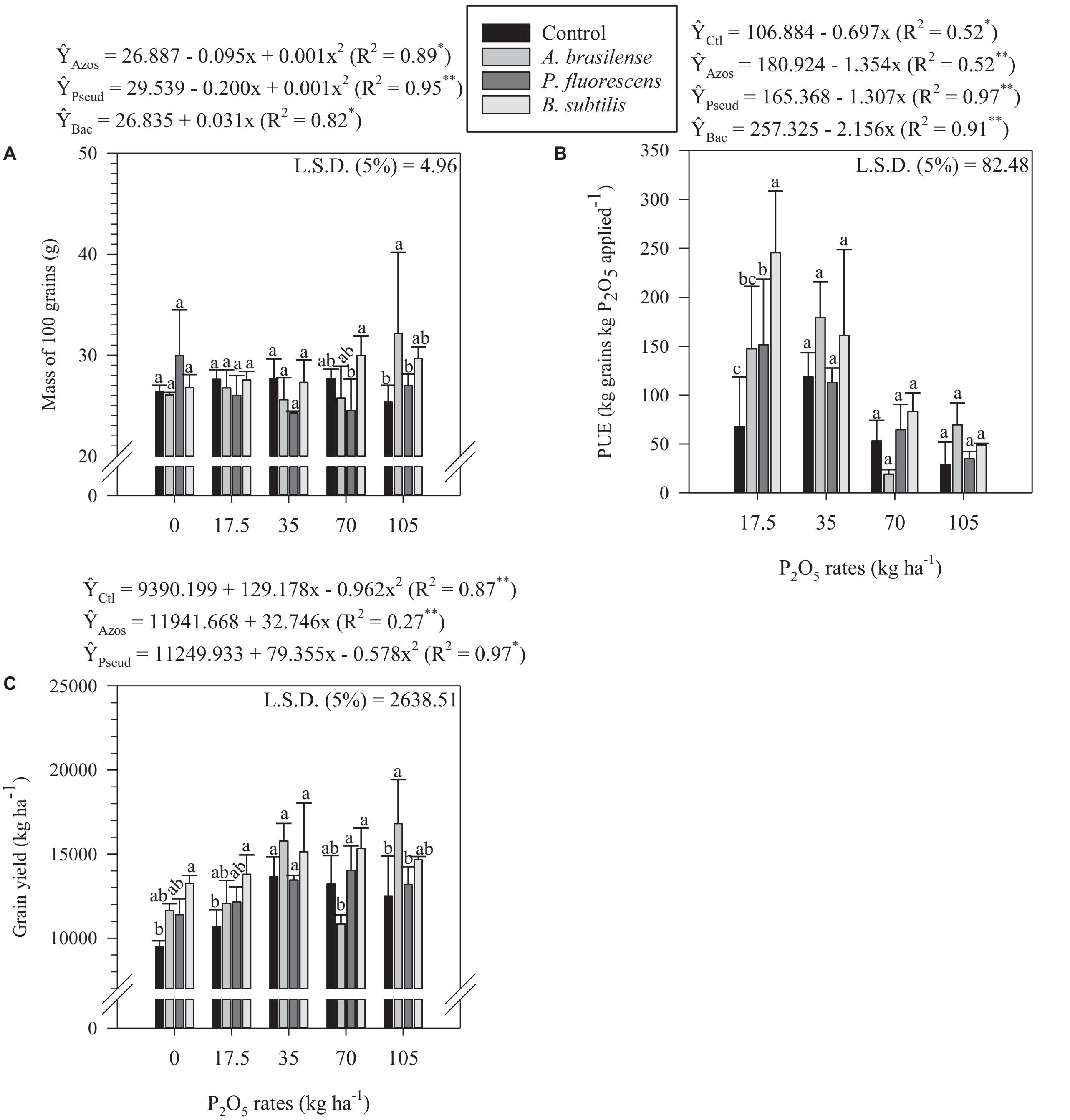

FIGURE 4 | Mass of 100 grains **(A)**, PUE **(B)**, and grain yield **(C)** as a function of the interaction between P_2O_5 rates and inoculations in corn crop. The letters correspond to a significant difference at 5% probability level ($p \leq 0.05$). Error bars indicate the standard deviation of the mean ($n = 4$). Ctl, Azos, Pseud e Bac refers to the control treatment and inoculations with *A. brasilense*, *P. fluorescens* and *B. subtilis*, respectively. L.S.D. (least significant difference), PUE (P use efficiency).

is limited as verified by the linear decreasing response in PUE to P_2O_5 application rates, regardless of PGPB inoculation. In addition, almost 80% of P content in soil can be fixed in forms unavailable to plants (White and Hammond, 2008; Zhang et al., 2016). Therefore, P application must be rational and optimized, since the increased P_2O_5 application rates results in increased losses and less utilization by cropping systems.

Although the response to PGPB inoculation under P_2O_5 application rates were different, the response to *B. subtilis* and *A. brasilense* inoculation associated with P_2O_5 rates were greater than control and *P. fluorescens* inoculation mainly when *P. fluorescens* inoculation was associated with P_2O_5 application rates. We verified this behavior on lower grain P uptake (associated with 35 and 105 kg P_2O_5 ha^{-1}), ear diameter (with

35 and 70 kg P_2O_5 ha^{-1}), mass of 100 grains (with 70 and 105 kg P_2O_5 ha^{-1}) and grain yield (with 105 kg P_2O_5 ha^{-1}) when *P. fluorescens* inoculation was performed. Positive responses to *B. subtilis* inoculation were verified mainly in the absence and application of 70 kg P_2O_5 ha^{-1}. In the absence of P_2O_5 application rates *B. subtilis* inoculation showed greater ear length, number of grains per row and grains per ear. With application of 70 kg P_2O_5 ha^{-1}, *B. subtilis* inoculation showed greater ear diameter and mass of 100 grains. In addition, when 17.5 kg P_2O_5 ha^{-1} was applied, *B. subtilis* inoculation showed greater PUE. Grain yield was also positive affected by *B. subtilis* inoculation in the absence of P_2O_5 application rates (increase of 39.5, 13.9, and 16.3%), 17.5 kg P_2O_5 ha^{-1} (increase of 29.1, 14.2, and 13.5%) and 70 kg P_2O_5 ha^{-1} (increase of 15.9, 41.3, and 9.2 compared to control, *A. brasilense* and *P. fluorescens*, respectively). Similarly, positive responses to *A. brasilense* inoculation were verified mainly with application of 35 and 105 kg P_2O_5 ha^{-1}. With application of 35 kg P_2O_5 ha^{-1}, *A. brasilense* inoculation showed greater ear length and ear diameter. When 105 kg P_2O_5 ha^{-1} was applied, *A. brasilense* showed greater mass of 100 grains and grain yield. Grain yield increased by 34.7, 27.7, and 14.8% compared to control, *P. fluorescens* and *B. subtilis* inoculated treatments, respectively, when *A. brasilense* was inoculated and 105 kg P_2O_5 $ha^{-1}$was applied. In addition, regardless of P_2O_5 application rates, *A. brasilense* inoculation showed greater P-resin in soil (increase of 19.7, 96.7, and 87.2%) and stem diameter (increase of 5.7, 12.1, and 12.1% compared to control, *P. fluorescens* and *B. subtilis*, respectively).

There was a dilution effect on P concentration as a function of *B. subtilis* and *A. brasilense* inoculation. It was observed that *B. subtilis* and *A. brasilense* inoculation promoted greater P uptake, optimizing the use of the absorbed P by corn plant. Our results showed that the inoculation with *B. subtilis* provided an increase of 9.7% for biomass P uptake (40.37 to 44.28 kg ha^{-1}, respectively) and 12.6% for grain P uptake (49.87 to 56.13 kg ha^{-1}, respectively) compared to control, regardless of P_2O_5 application rates. Also, the inoculation with *A. brasilense* provided an increase of increase of 10.% for biomass P uptake (40.37 to 44.42 kg ha^{-1}) and 21.3% for grain P uptake (49.87 to 60.50 kg ha^{-1}, respectively) compared to control, regardless of P_2O_5 application rates. The exact mechanisms underlying the *B. subtilis* and *A. brasilense* effect on corn growth was not evaluated in the present study, however, it is very likely that the improvement in P availability and uptake reflecting on improved PUE and grain yield by corn verified in this study is associated with the well-known ability of *A. brasilense* and *B. subtilis* to promote plant growth (Fukami et al., 2017; Jang et al., 2018; Martins et al., 2018; Posada et al., 2018; Salvo et al., 2018). The increase on PUE and corn yield as a function *B. subtilis* and *A. brasilense* inoculation compared to control was, on average, equivalent to 100.5 and 54.6% for PUE and 17.4 and 12.8% for grain yield, respectively, regardless of P_2O_5 application rates. Similar results with *B. subtilis* and *A. brasilense* inoculation were reported with greater corn yield between 3.8 to 34% in inoculated plants compared with non-inoculated plants (Kuan et al., 2016; Müller et al., 2016; Traoré et al., 2016; Ahmad et al., 2019; Galindo et al., 2019b).

The bacteria *B. subtilis* is well known to possess properties of plant growth promotion, phosphate solubilization, phytopathogen inhibition and heavy metal absorption (Traoré et al., 2016; Rekha et al., 2017; Muñoz-Moreno et al., 2018; Prakash and Arora, 2019). In addition, the strains Ab-V5 and Ab-V6 of *A. brasilense* share the genes related to the synthesis of auxins (Hungria et al., 2018). Also, *Azospirillum* spp. inoculation is related to nutrient availability increase (Galindo et al., 2018b) and BNF (Pankievicz et al., 2015). This growth promotion mechanisms might have improved the ability of the plants to more efficiently explore the soil and uptake P, as indicated in previous studies using *B. subtilis* and *A. brasilense* (Traoré et al., 2016; Martins et al., 2018; Prakash and Arora, 2019; Zeffa et al., 2019). According to Cormier et al. (2013), two strategies may be devised for nutrient use efficiency improvement: increasing the yield at a constant nutrient supply and/or maintaining high yield when reducing nutrient supply. Therefore, the results of our research show that inoculation with *B. subtilis* and *A. brasilense* may be a potential strategy to help improving PUE. It is not unusual that PGPB displays several different plant-beneficial properties (Bruto et al., 2014; Vacheron et al., 2016), which is thought to provide higher positive effects on the plant (Bashan and de-Bashan, 2010). This is expected to take place because (i) the effects of different modes of action may add-up quantitatively, or (ii) it could ensure that at least one mode of action is expressed in particular environmental conditions (Vacheron et al., 2016). Indeed, the most effective PGPB are typically multi-function strains (Almario et al., 2014).

The inoculation with *P. fluorescens* had little quantifiable effect on P uptake, corn development and grain yield. However, *P. fluorescens* has been reported to favor biological control of plants, production of natural antibiotics and protective effect against secondary soil phytopathogens (Garrido-Sanz et al., 2016), phosphate solubilization (Oteino et al., 2015) and N_2 fixation (Vacheron et al., 2016). Also, *P. fluorescens* resulted in 35.3 and 7.8% increase in PUE and corn grain yield compared to control treatment, respectively, regardless of P_2O_5 application rates. Evidently, there is still great divergence in the use of PGPB in corn and other grasses due to the variable results with inoculation. However, it is important to highlight the importance of research on the subject and the potential of using this technology, mainly because it is easy to apply, is low cost, and has a great potential to promote plant growth.

CONCLUSION

Inoculation with *B. subtilis* and *A. brasilense* associated with P_2O_5 application rates were found to increase P uptake, benefiting productive components development, leading to an improved PUE, and greater corn grain yield. Yield increased by 39.5, 29.1, and 15.9% when plants were inoculated with *B. subtilis* in the absence of P_2O_5 application rates, associated with 17.5 and 70 kg P_2O_5 ha^{-1}, respectively. Inoculation with *A. brasilense* increased grain yield by 34.7% when 105 kg P_2O_5 ha^{-1} was applied, showing the potential for improve PUE by the *B. subtilis* and *A. brasilense* inoculation, positively reflecting on corn grain yield. The inoculation with *P. fluorescens* had small effects on P uptake, plant development and grain yield, however, resulted in 35.3 and 7.8% increase on PUE and corn grain yield compared to control. Therefore, studies conducted under biotic or/and abiotic conditions are necessary to better understand the role of PGPB, inoculated alone or in combination as the co-inoculated method.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

FG and MT wrote the manuscript, with contributions from NP. MT, RG, and ED corrected and improved the manuscript. NP, FG, PR, and EM conducted the samplings and data collection. NP did the analysis, with the support of MT and FG.

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