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Full Length Article

Seed Inoculation with *Pseudomonas fluorescens* Promotes Growth, Yield and Reduces Nitrogen Application in Maize

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Abstract

Plant growth-promoting bacteria (PGPB) improve plant nutrition, growth, resistance to stresses and yield, having long been employed to inoculate crops worldwide. *Pseudomonas fluorescens* exerts acknowledged PGPB activity towards several crops, inducing systemic resistance to diseases, plant tolerance to adverse conditions, and promote yield. In present study, inoculation of maize seeds with *P. fluorescens* was compared with *Azospirillum brasilense*, a commercial PGPB inoculant, with a concomitant 25% reduction in the amounts of N fertilizer recommended. Six field experiments were carried out in distinct geographic regions of Brazil, where maize is grown under standard or high technological level, with different expectations regarding grain yield. The results demonstrated that *P. fluorescens* promoted plant growth and yield at both levels, increasing plant biomass accumulation by 24 and 20%, relative to the non-inoculated control, at standard or high levels, respectively. The grain yield increased by 29 and 31%, relative to the non-inoculated control, under standard and high levels of technology, respectively. Under both situation, plant growth and grain yield improved by *P. fluorescens* was equivalent to the application of 100% of the recommended N fertilizer, even when the amount of N fertilizer applied to the crop was reduced by 25%, without compromising yield. For the first time, the successful utilization of *P. fluorescens* for growth-promotion of maize, in Brazil, with a concomitant reduction of the need for N fertilizer, resulting in profitable yields with reduced costs and environmental impacts was reported.

Keywords: Plant growth-promotion; Grain yield; Soil fertility; Conventional tillage; No-tillage

Introduction

The inoculation of agricultural crops with plant growth-promoting bacteria (PGPB) is a consolidated biotechnological practice worldwide (Bashan *et al.*, 2014; Hungria and Mendes, 2015). PGPB comprise of a heterogeneous group of beneficial bacteria commonly found in the rhizosphere, on the root surface or associated to it, which are capable of enhancing the growth of plants and protecting them from biotic and abiotic stresses (Souza *et al.*, 2015). Such stimuli may result from nutrient mobilization, production of plant growth regulators, control or inhibition of plant pathogens and bioremediation of soils, among other mechanisms (Bashan *et al.*, 2012; Nehra and Choudary, 2015; Yasmin *et al.*, 2016).

The bacterial genus *Pseudomonas* harbors several species with acknowledged activity as PGPB (Antoun and Prévost, 2005). *P. fluorescens* is probably the most extensively studied species of this PGPB genus, showing great ability to adapt to plant rhizospheres (Rainey, 1999; Landa *et al.*, 2002; Botelho and Hagler, 2006) and promote plant growth by a diversified array of mechanisms (David *et al.*, 2018). For example, *P. fluorescens* is able to bioprime sunflower (*Helianthus annuus* L.) seeds, improving seed invigoration and seedling growth (Moeinzadeh *et al.*, 2010), controls damping-off of cotton (*Gossypium hirsutum* L.) (Ardakani *et al.*, 2010) and induces systemic resistance to viral diseases in tobacco (*Nicotiana tabacum* L.) (Maurhofer *et al.*, 1994) and bananas (*Musa* spp.) (Kavino *et al.*, 2008).

In addition, inoculation with *P. fluorescens* results in yield increase of wheat (*Triticum aestivum* L.) (Naiman *et al.*, 2009), enhances plant biomass production by *Catharanthus roseus* under water deficit stress (Jaleel *et al.*, 2007) and increases germination, seedling growth and yield of maize (*Zea mays* L.) (Gholami *et al.*, 2009). Despite all the positive reports on the utilization of *P. fluorescens* as a

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PGPB worldwide, little is known about its action and viability under Brazilian conditions, where there are no commercial inoculants available carrying the species. So far, only *Azospirillum brasilense* is successfully employed in Brazil to promote the growth and yield of field-grown maize and wheat (Hungria *et al.*, 2010), growth and biomass production by *Brachiaria* spp. in degraded pastures (Hungria *et al.*, 2016) and nodulation and yield when co-inoculated with rhizobia in soybean (*Glycine max* [L.] Merr.) and common bean (*Phaseolus vulgaris* L.) (Hungria *et al.*, 2013). In this study, the successful utilization of a new commercial product containing *P. fluorescens* to inoculate field-grown maize, resulting in increased yield with a significant reduction in the need for N-fertilizer under Brazilian conditions is reported.

Materials and Methods

Experimental Sites and Institutions

The experiments were carried out from 2012 to 2016, by two independent institutions, at different experimental sites, representative of the maize growing areas of Brazil. The team of Empresa Brasileira de Pesquisa Agropecuária – Embrapa Soja performed four experiments in the States of Goiás and Paraná and the team of Universidade Estadual do Centro-Oeste - UNICENTRO carried out two others, in the State of Paraná. Relevant information about the experimental sites is presented in Table 1.

Seed Inoculants

The inoculant products employed in this study are produced and marketed by Total Biotecnologia Indústria e Comércio S/A, from Curitiba, State of Paraná, Brazil, who kindly provided the products for our tests. Audax™ is a liquid formulation containing the strain CCTB 03 (CNPSo 2719) of *Pseudomonas fluorescens*. Strain CCTB 03 (CNPSo 2719) was selected as a promising PGPB by Total Biotecnologia Indústria e Comércio S/A in a screening of *P. fluorescens* isolates obtained from a private property to promote growth of maize (data not shown). The strain is currently deposited at the Culture Collection of Diazotrophic and Plant Growth-Promoting Bacteria of Embrapa Soja (WFCC Collection # 1213, WDCM Collection # 1054). The Audax™ formulation contains 1 x 10^8 colony forming units (CFU) mL^{-1} and the recommended dose is 100 mL 60,000 $seeds^{-1}$.

AzoTotal™ is a liquid formulation containing strains Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084) of *A. brasilense* at the concentration of 2 x 10^8 CFU mL^{-1}, with a recommended dose of 100 mL 60,000 $seeds^{-1}$. It is currently registered, recommended and available in the market as a commercial product containing PGPB for wheat, maize, *Brachiaria* spp. and co-inoculation of soybean and common bean. It has been included in this study as a control PGPB product.

Inoculants were applied directly to seeds at the recommended doses, according to the manufacturers' instructions. Seed inoculation was done in the shade, shortly before sowing.

Treatments, Experimental Design and Agronomical Procedures

The experiments conducted by Embrapa Soja were planted in areas under conventional soil tillage, whereas the experimental area of UNICENTRO was managed under a no-tillage system with oats (*Avena sativa* L.) as the previous crop. At each location, soil samples were collected from the top 20 cm soil layer two months prior to sowing for chemical and physical analyses (Hungria *et al.*, 2015). Soil chemical and physical properties are presented in Table 2. In the case of Embrapa Soja, when necessary, according to soil pH, lime was applied to the soil 40 days before planting to increase base saturation to 70%, in Paraná and 50% in Goiás (Lopes *et al.*, 1991; Soja, 2013).

At all sites, four treatments were implanted: *i*) non-inoculated control, with no side dress N fertilizer; *ii*) non-inoculated control, with 100% of the recommended dose of side dress N fertilizer; *iii*) seed inoculation with AzoTotal™, with 75% of the recommended side dress N fertilizer; and *iv*) seed inoculation with Audax™, with 75% of the recommended side dress N fertilizer.

Embrapa Soja experiments were set up under standard technological level, more representative of the general conditions for growing maize in Brazil, where lower amounts of basal and side dress fertilizers were applied, no seed treatment with agrichemicals was performed before sowing and plant population in the field was lower. Basal fertilization consisted of 300 kg ha^{-1} of the 08-20-20 N-P-K. A side dress of 90 kg N ha^{-1} (treatment *ii*, 100%N) and 67.5 kg N ha^{-1} (treatments *iii* and *iv*, 75%N) was performed at the V4 (four fully developed leaves) stage. Crop cultural and sanitary management followed the guidelines established by Embrapa Milho e Sorgo for maize (Sorgo, 2011). Experiments were not irrigated. All plots had a minimum area of 24.5 m^2, with rows spaced by 0.9 m. In addition, plots were separated by 2 m terraces to avoid cross contamination due to surface runoff of bacteria and fertilizers.

UNICENTRO experiments were set up under high-technological level, very specific for the geographic region where the experiments were carried out, consisting of higher levels of basal and side dress fertilization, seed treatment with agrichemicals before sowing, highly productive maize hybrids and increased plant population in the field. The desiccation of the experimental area was accomplished by applying Glyphosate, at 720 g active ingredient ha^{-1}. All seeds were treated before planting with Standak (Fipronil) + Cruiser (Tiametoxam) + Maxim XL (Metalaxil-M + Fludioxonil), at 60 + 120 + 100 mL 60,000 $seeds^{-1}$. Plots of all treatments received basal fertilization consisting of 49.4 kg N ha^{-1}, 133 kg P_2O_5 ha^{-1}, and 38 kg K_2O ha^{-1} and a side-dress fertilization with 100 kg K_2O ha^{-1} at the V3 (three fully developed leaves) stage. Plots of treatment *ii* (100% N) received two side-dress applications of 100 kg N ha^{-1} (100% N) each, at the V3 and V5 (five fully developed leaves) stages. For treatments *iii* and *iv*, plots received two side-dress applications of 75 kg N ha^{-1} each, performed at the V3 and V5 stages. Post-emergence sprays of atrazine + tembotrione, at 2,250 and 75.6 g active ingredient ha^{-1}, respectively, were performed for weed control. No control of pests and foliar diseases was done. All plots had ten rows. All rows were 6 m-long, and were separated from one another by 0.425 m.

At all locations, experiments were set up under a completely randomized block design with six replicates.

Sampling Procedures

In the experiments conducted by Embrapa Soja, five plants were randomly collected from each plot between 44 and 57 days after sowing (DAS), to evaluate vegetative growth. Shoots were rinsed and allowed to dry at 50ºC until constant weight and plant biomass was determined. Dried shoots were then ground for determination of total N accumulation after sulfuric digestion followed by distillation, according to the kjeldahl method (Hungria *et al.*, 2015). Grain yield was determined after harvesting a minimum area of 7.2 m^2 from each plot at physiological maturity. All grains were removed from the ears, cleaned, weighed and the moisture was corrected to 13% before estimating grain yield on a kg ha^{-1} basis.

At UNICENTRO, samples were collected from the four central rows of each plot, allowing 1 m from each end as border, and analyzing an area of 6.8 m^2. When the grains in the ears had reached the ¾ of the milk line stage (R5 stage), 10 plants were collected from each plot to determine plant biomass, as described above for the experiments conducted by Embrapa Soja, at the silage point. At physiological maturity, all ears were collected and thrashed and the grain weight at 14% moisture was employed to estimate grain yield in kg ha^{-1}. The weight of 1,000 grains was estimated from a sample of 300 grains from each plot.

Statistical Analyses

All data from the experiments carried out in Cachoeira Dourada, Palmeira, Ponta Grossa, and Londrina were subjected to tests of normality and homogeneity of the variances and an analysis of variance (ANOVA) was performed. When significant treatment effects were detected by the ANOVA, means were compared by Duncan's test ($P < 0.05$).

In the case of UNICENTRO (Guarapuava), data were subjected to tests of normality and homogeneity of the variances and an analysis of variance (ANOVA) was performed. When significant treatment effects were detected by the ANOVA, means were compared by Tukey's test ($P < 0.05$).

Results

Experiments Carried Out in Cachoeira Dourada, Palmeira, Ponta Grossa and Londrina

Plant biomass accumulation responded significantly to all treatments relative to the non-inoculated, no N control in the experiment planted in Cachoeira Dourada, where seed inoculation with AzoTotal™ + 75% N or Audax™ + 75% N improved plant growth better than plants that received the full dose of N (Table 3). The same trend was observed in Palmeira, whereas plants did not respond to any treatment in Ponta Grossa, and showed a slight but not significant response to the full dose of N fertilizer in Londrina (Table 3). Interestingly, when positive responses were observed, plant biomass increases promoted by inoculation with either AzoTotal™ + 75% N or Audax™ + 75% N were superior to those promoted by the full dose of N fertilizer (Table 3).

In two (Cachoeira Dourada and Palmeira) out of the four experiments, N accumulation in the shoots was significantly increased by inoculations, relative to the non-inoculated, no N control, turning out comparable to when plants received the full dose of N fertilizer (Table 3). The same trend, but with no significant differences was observed in Ponta Grossa and Londrina. Taking only the absolute values into account, in Cachoeira Dourada, Palmeira and Ponta Grossa, plants from seeds inoculated with either AzoTotal™ + 75% N or Audax™ + 75% N accumulated more N in the shoots than when 100% N fertilizer was applied (Table 3).

Grain yield was significantly increased by inoculation with either AzoTotal™ + 75% N or Audax™ + 75% N, or 100% N, relative to the non-inoculated, no N control, in three out of the four experiments (Table 3). The results obtained from seed inoculation with Audax™ + 75% N (29% average increase across all four locations) were quite impressive. In Ponta Grossa and Londrina, increases in grain yield promoted by the inoculation with Audax™ + 75% N were significantly higher than those obtained with the inoculation of AzoTotal™ + 75% N and with the application of the full (100% N) dose of side dress N fertilizer (Table 3).

Experiments Carried out in Guarapuava

Plant biomass, 1000-grain weight, and grain yield were significantly affected by all treatments, relative to the non-inoculated no N control, in both crop seasons, except in the 2014/2015 season, when only the application of 100% of the recommended N fertilizer and inoculation with Audax™ + 75% N significantly improved plant

Table 1: Description of the experimental sites and maize hybrid planted at each location

Experimental Site	Geographic Coordinates♥	Elevation♦ (m)	Climate Type♣	Cropping Season	Soil Type♠	Hybrid Planted
Embrapa Soja						
Cachoeira Dourada	18º29'S, 49º28'W	459	Aw	2012/2013	Typic Hapludox	DOW 2B 707 HX
Palmeira	25º25'S, 50º50'W	865	Cfb	2012/2013	Typic Hapludox	DOW 2B 707 HX
Ponta Grossa	25º13'S, 50º10'W	880	Cfb	2014/2015	Typic Hapludox	DKB-350-PRÓ II
Londrina	23º11'S, 51º11'W	620	Cfa	2015/2016	Rhodic Eutrudox	DKB-350-PRÓ II
UNICENTRO						
Guarapuava	25º30'S, 51º47'W	889	Cfb	2014/2015	Typic Hapludox	DKB-290-PRÓ II
Guarapuava	25º30'S, 51º47'W	889	Cfb	2015/2016	Typic Hapludox	AS 1656 PRO III

♥Latitude and longitude
♦Above average sea level
♣According to Köppen's [Alvares *et al.*, 2013] Classification
♠According to the U.S.A. Soil Taxonomy System

Table 2: Chemical properties and granulometry analyses of the soils at the experimental sites

	pH	Al	H + Al	K	Ca	Mg	P	C	SB♦	BS♠	Granulometry (%)		
	($CaCl_2$)	------------------------- $cmol_c\ dm^{-3}$ ----------------					$mg\ dm^{-3}$	$g\ dm^{-3}$	$cmol_c\ dm^{-3}$	%	Clay	Silt	Sand
Sites♣							2012 - 2013						
C. Dourada	5.4	0	7.89	0.15	2.02	1.30	0.80	30.50	3.47	30	57.8	18.2	24.0
Palmeira	4.6	0.26	3.07	0.37	3.55	1.73	1.71	18.55	5.65	65	58.4	15.7	25.9
							2014-2015						
P. Grossa	4.9	0.07	4.90	0.44	2.95	1.06	25.70	18.00	4.45	52	45.8	5.2	49.0
Guarapuava	5.3	0	4.56	0.55	4.78	2.00	3.76	25.31	7.33	62	60.1	8.4	31.6
							2015 – 2016						
Londrina	5.4	0	2.70	0.86	4.52	2.17	19.40	11.80	7.55	74	71.0	8.2	20.8
Guarapuava	5.2	0	4.71	0.58	4.70	2.02	3.21	25.77	7.30	61	62.0	7.6	30.4

♣ C. Dourada = Cachoeira Dourada; P. Grossa = Ponta Grossa; ♦ SB = Sum of Bases; ♠ BS = Base Saturation = [(K + Ca + Mg)/ T_{cec}] × 100, where T_{cec} = K + Ca + Mg + total acidity at pH 7 (H + Al)

Table 3: Plant biomass and shoot nitrogen accumulation during growth (ca. 50 days after sowing), and grain yield of maize at the stage of plant maturity in four independent experiments conducted by Embrapa Soja

	Plant biomass								Total N in the shoots								Grain yield							
TRT♦	---------- $g\ pl^{-1}$ ----------								---------- $mg\ N\ pl^{-1}$ ----------								---------- $kg\ ha^{-1}$ ----------							
	CACH♥	%♣	PALM	%	PGRO	%	LOND	%	CACH	%	PALM	%	PGRO	%	LOND	%	CACH	%	PALM	%	PGRO	%	LOND	%
CON	29.4 b♠		49.1^{ns}		43.9^{ns}		25.0^{ns}		678 b		927 b		1066^{ns}		639 b		5427b		7294 b		5972c		7680 c	
NIT	36.0 a	22	53.5	9	40.8	-	29.7	19	793 ab	17	1279 a	38	1226	15	853 a	34	6513ab	20	8420 a	15	7723b	29	8711 b	13
AZ+N	41.1 a	40	58.3	19	40.9	-	24.7	-	900 a	33	1398 a	51	1255	18	616 b	-	6658ab	23	8850 a	21	7230b	21	9020 b	17
AD+N	40.3 a	37	55.1	12	40.4	-	25.1	=	874 a	29	1300 a	40	1292	21	748 ab	17	6831a	26	9115 a	25	8306a	39	9760 a	27

♦ Treatments (TRT) are: CON – non-inoculated, no N control; NIT – 100% N fertilizer; AZ+N - AzoTotal™ + 75% N fertilizer; AD+N - Audax™ + 75% N fertilizer
♥ Experimental locations are: CACH – Cachoeira Dourada; PALM – Palmeira; PGRO – Ponta Grossa; LOND - Londrina
♠ Means (n = 6) followed by the same letter in the column are not significantly different according to the comparison by Duncan's ($P < 0.05$) test; ns denotes that no significant differences among means were detected
♣ Numbers refer to increase (%) in parameter relative to the control (non-inoculated, no N) treatment; - indicates a decrease, and = indicates neither increase or decrease

Table 4: Plant biomass, 1,000-grain weight and grain yield of maize at the stage of plant maturity in the experiments conducted at two independent growth seasons by UNICENTRO

	Plant biomass				1,000-grain weight				Grain yield			
Treatment♦	-------------- $kg\ ha^{-1}$ --------------				---------- g ----------				---------- $kg\ ha^{-1}$ ----------			
	2014/2015	%♣	2015/2016	%	2014/2015	%	2015/2016	%	2014/2015	%	2015/2016	%
Control	16,058 b♠		35,950 b		296.3 b		395.3 b		8,861 b		11,483 b	
100% N	19,509 a	21	44,925 a	25	364.9 a	23	422.5 a	7	12,710 a	44	13,215 a	15
Azo+75%N	18,255 ab	14	43,228 a	20	356.6 a	20	422.7 a	7	12,636 a	43	13,050 a	14
Aud+75%N	19,452 a	21	43,148 a	20	356.2 a	20	423.7 a	7	12,913 a	46	13,321 a	16

♦ Treatments are: Control – non-inoculated, no N control; 100% N – 100% N fertilizer; Azo+75%N - AzoTotal™ + 75% N fertilizer; Aud+75%N - Audax™ + 75% N fertilizer
♠ Means (n = 6) followed by the same letter in the column are not significantly different according to the comparison by Tukey's ($P < 0.05$) test
♣ Numbers refer to increase (%) in parameter relative to the control (non-inoculated, no N) treatment

biomass relative to the non-inoculated control (Table 4). Inoculation with either product resulted in increases in plant biomass accumulation comparable to those obtained in response to the application of 100% N fertilizer (Table 4). Plant biomass was superior in the second season because of a difference in sowing dates between seasons. In 2014/2015, the crop was sown after the ideal dates for planting maize in the geographic region concerned, whereas in 2015/2016 the crop was sown right on time, meaning that plants had more days with ideal growth conditions in a region with a humid subtropical (Cfb) climate.

Inoculation with either AzoTotal™ + 75%N or Audax™ + 75%N, as well as application of 100% N significantly increased the weight of 1000 grains of maize, reflecting, consequently, in grain yield (Table 4), which was also positively and significantly affected by the three treatments. In 2014/2015, inoculation with Audax™ + 75%N promoted a 46% increase in grain yield relative to the non-inoculated, no N control (Table 4). In general, the crop responded equally well to both inoculation with either product or to the full dose of N fertilizer applied.

Discussion

The association of plants with beneficial micro-organisms has long been known and exploited in agriculture and many different methods have been employed to promote such associations (Bashan, 1998). The most successful example of such practices is the worldwide utilization of rhizobial inoculants for legumes, but more recently other bacteria, collectively known as plant growth-promoting bacteria (PGPB) have been given more attention and developed into inoculants to be employed in agriculture.

Plant growth in the soil is under the influence of both abiotic and biotic factors and, especially, of actions that take place in the rhizosphere (Saharan and Nehra, 2011). Micro-organisms that colonize the rhizosphere, especially bacteria, may exert various effects on plant growth, ranging from pathogenicity to growth promotion, either directly or indirectly. Representatives of the bacterial genera *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Burkholderia* and *Bacillus*, among others, have been shown to inhabit plant rhizospheres and behave as PGPB (Kloepper *et al.*, 1989; Glick, 1995; Joseph *et al.*, 2007).

In this study, the plant growth-promoting performance of Audax™, a newly developed, commercial formulation containing *P. fluorescens* strain CCTB 03 (CNPSo 2719) was evaluated under field conditions at two different levels of technology for the maize crop in different geographic regions of Brazil. The standard level of technology mentioned is defined by no seed treatment with agrichemicals before sowing, lower levels of basal and side dress fertilization and average cultural practices for the maize crop. This level of technology is quite representative of the average growth conditions for the maize crop in Brazil. On the other hand, the high level of technology employed is characterized by heavier basal and side dress fertilization, seed treatment with agrichemicals before sowing, intensive cultural practices, and is representative of maize growth in an extremely favorable micro region of the State of Paraná, where very high yields are regularly expected and obtained.

The growth-promoting ability of Audax™ was compared to AzoTotal™, another commercial product, containing two strains of *A. brasilense* (Ab-V5 and Ab-V6), which is currently available and recommended as a PGPB-based inoculant for maize in Brazil. In addition, both products were tested in a situation of reduced nitrogen (N) fertility, where plants received 75% of the recommended dose of N fertilizer as side dress during the growth cycle.

The results showed that under both levels of technology employed for maize growth in present experiments, the *P. fluorescens* in Audax™ promoted plant growth, estimated as plant biomass, N accumulation and grain yield of the maize crop, thus confirming its potential as PGPB. The beneficial effects of Audax™ were more pronounced when plants were grown under standard levels of technology, as demonstrated in the experiments conducted by Embrapa Soja. The impressive grain yield promotion by inoculation with Audax™ in Ponta Grossa and Londrina illustrate the potential of *P. fluorescens* as a PGPB. Under lower levels of fertility, gains of the magnitudes of 39% (Ponta Grossa) and 27% (Londrina) relative to the non-inoculated control could be obtained, and such gains were even more pronounced than when 100% N fertilizer was employed.

In fact, the efficiency of *P. fluorescens* as a PGPB may be related to soil fertility levels, as demonstrated by Shaharoona *et al.* (2008), who observed that the efficacy of some strains of *P. fluorescens* as PGPB for wheat decreased with increasing rates of NPK fertilizer added to the soil. In such situations, the PGPB may improve nutrient uptake from the soil, as demonstrated for *Azotobacter* spp., *Azospirillum* sp., as well as other species of *Pseudomonas* (Zaefarian *et al.*, 2012; Sultana *et al.*, 2016). The soils at both experimental stations in Ponta Grossa and Londrina have been extensively cropped and taking into account that the areas were maintained under conventional soil tillage, as opposed to the no-tillage practices adopted in Guarapuava, lower levels of natural soil fertility can be expected.

When plants were grown under high levels of technology, as exemplified by the experiments conducted by UNICENTRO, all parameters tended to respond better to N fertilizer, but yield increases of a magnitude above 40% could still be observed when N fertilization was reduced by 25%. Shaharoona *et al.* (2006) reported that *P. fluorescens* strains producing the enzyme 1-aminocylopropane-1-carboxylate (ACC)-deaminase perform better in the presence of reduced levels of N, especially NO_3, in the soil. Glick *et al*. (1998) proposed that PGPB strains possessing ACC-deaminase can hydrolyze ACC, the precursor of ethylene in higher plants, producing α-ketobutyrate and ammonia which, in turn, can promote plant growth. Inoculants containing such strains can be effective to improve the growth and yield of inoculated plants, even in the presence of optimum levels of N fertilizers. Strain CCTB 03 produces ACC-deaminase (data not shown), what may explain its behavior under our experimental conditions.

The *P. fluorescens* has great ability to adapt to plant rhizospheres (Rainey, 1999; Landa *et al.*, 2002; Botelho and Hagler, 2006), for example, in the effective biocontrol of wilt caused by the fungus *Fusarium oxysporum* f. spp. *ciceris* in chickpea (*Cicer arietinum* L.) (Vidhyasekaran and Muthamilan, 1995), or in alleviation salt stress in maize (Nadeem *et al.*, 2007). In addition, it promotes the growth of some plant species under water deficit stress (Jaleel *et al.*, 2007; Zahir *et al.*, 2008). The causes of the success of *P. fluorescens* as a PGPB are poorly understood, but it has been demonstrated (Gal *et al.*, 2012) that the species carries many genes that are expressed only in the rhizosphere, which may be determinants to its environmental fitness.

The mechanisms responsible for the PGPB activity of strain CCTB 03 in Audax™ were not determined in present study, but results demonstrate that it is possible to reduce the amount of N fertilizer applied to the crop when Audax™ is employed for seed inoculation. On the average of six experiments, a 29.8% increase in the grain yield of maize could be obtained in response to seed inoculation with PGPB *P. fluorescens* CCTB 03, present in Audax™, even when side dress N fertilization was reduced by 25%.

Taking into account the average recommendation of 90 kg N ha^{-1} side dress fertilization for the maize crop, the utilization of the innovative biotechnology described in this study could represent a reduction of 22.5 kg N ha$^{-1.}$ The total area planted with maize in Brazil in the 2017/2018 crop season was approximately 12 million hectares. The utilization of the Audax™ could contribute to reduce the application of about 270,000 tons of N (approximately 560,000 tons of urea) for the maize crop in Brazil, thus helping to reduce production costs and to mitigate the negative effects related to CO_2 release to the atmosphere by agricultural activities (Hungria *et al.*, 2016).

This innovative biotechnology brings an alternative to help reduce costs and environmental problems related to excessive use of fertilizers, and yet obtain optimum yields by maize grown under standard or high technological levels in Brazil.

Conclusion

Audax™ contains a strain of *P. fluorescens* for the inoculation of maize seeds prior to sowing in Brazil that is capable of promoting plant growth and yield under different levels of technology applied to the maize crop. In any case, the amount of N fertilizer applied to the crop may be reduced by 25%, without compromising yield.

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