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Maize response to inoculation with strains of plant growth-promoting bacteria

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phytohormones

A B S T R A C T

The aim of this study was to evaluate the response of maize to inoculation with strains of plant growth-promoting bacteria (PGPB) in two cultivation years. The experiment was set in a randomized block design with four replicates in two cultivation years (2012/13 and 2013/14). The treatments consisted of PGPB inoculation: control (without N and without inoculation); 30 kg of N ha^{-1} at sowing (N1); 160 kg of N ha^{-1} (N1 + 130 kg of N ha^{-1} as top-dressing); N1 + *A. brasilense*, Ab-V5; N1 + *A. brasilense*, HM053; N1 + *Azospirillum* sp. L26; N1 + *Azospirillum* sp. L27; N1 + *Enhydrobacter* sp. 4331; N1 + *Rhizobium* sp. 8121. Basal stem diameter, plant height, leaf area, shoot dry matter and yield were evaluated. The strain of *Rhizobium* sp. 8121and the isolate *Azospirillum* sp. L26 associated with 30 kg of N ha^{-1} at sowing promoted yields equivalent to that of the N fertilization of 160 kg ha^{-1}, demonstrating the potential to be used in the inoculation of maize seeds.

Palavras-chave:
Zea mays L.
Azospirillum sp.
Rhizobium sp.
fito-hormônios

Resposta do milho à inoculação de estirpes de bactérias promotoras do crescimento de plantas

R E S U M O

Neste trabalho objetivou-se avaliar a resposta do milho à inoculação de estirpes de bactérias promotoras do crescimento de plantas (BPCP's) em dois anos de cultivo. O experimento foi conduzido em delineamento experimental de blocos ao acaso com quatro repetições em dois anos de cultivo (2012/13 e 2013/14). Os tratamentos foram constituídos pela inoculação de BPCP's: controle (sem N e sem inoculação); 30 kg ha^{-1} N na semeadura (N1); 160 kg ha^{-1} N (N1 + 130 kg ha^{-1} N em cobertura); N1 + *A. brasilense*, Ab-V5; N1 + *A. brasilense*, HM053; N1 + *Azospirillum* sp., L26; N1 + *Azospirillum* sp., L27; N1 + *Enhydrobacter* sp. 4331; N1 + *Rhizobium* sp. 8121. Diâmetro basal do colmo, altura da planta, área foliar, massa seca da parte aérea e produtividade foram avaliados. A estirpe de *Rhizobium* sp. 8121 e o isolado de *Azospirillum* sp. L26 associados à adubação com 30 kg ha^{-1} N na semeadura proporcionaram produtividades equivalentes à adubação com 160 kg ha^{-1} N demonstrando potencial para serem utilizadas na inoculação de sementes de milho.

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Introduction

Maize (*Zea mays* L.) is the main cereal produced in Brazil, with total cultivated area in the 2014/15 season of 15,207 thousand hectares and mean yield of 5,168 kg ha^{-1} (CONAB, 2015). Various studies have been conducted under different conditions of soil, climate and cultivation system, showing general response of maize to nitrogen (N) fertilization (Ferreira et al., 2009; Gava et al., 2010; Santos et al., 2010). However, the production process and the need to import N fertilizers make their acquisition more expensive. In this context, one alternative aiming to optimize their use in the maize crop consists in the association with plant growth-promoting bacteria (PGPB)

The growth-promoting effect caused by the inoculation of PGPB results from the production of phytohormones by the bacteria, as well as other mechanisms of growth promotion, such as the stabilization of phosphates, biological N fixation (BNF) and the increase in the activity of specific enzymes (Okon & Vanderleyden, 1997; Rodriguez & Fraga, 1999; Radwan et al., 2004).

Lana et al. (2012) report positive effect of inoculation with *A. brasilense* in the absence of N as top-dressing, with increment of 15% in maize yield; other studies also highlight the effect of maize growth promotion caused by other PGPB genera, such as: *Sphingomonas* spp. (Pedrinho et al., 2010), *Burkholderia* spp. (Alves et al., 2015), *Herbaspirillum* spp. (Dotto et al., 2010; Araújo et al., 2013) and *Rhizobium* spp. (Hahn et al., 2014).

The selection of efficient strains is a key factor for the success of inoculation (Hungria et al., 2010). Reis Júnior et al. (2008) isolated different strains of *Azospirillum* spp. and *Herbaspirillum* spp. from the root system of maize varieties and observed variable potential regarding the production of auxins (IAA) and BNF, selecting strains that promoted greater plant growth for later studies in greenhouse and at the field. Thus, the present study aimed to evaluate the response of maize to the inoculation of PGPB strains.

Material and Methods

The experiment was carried out at the field, in the summer seasons of 2012/13 and 2013/14, at the Experimental Station of the Agronomic Institute of Paraná - IAPAR, in Pato Branco-PR, Brazil (25° 07' S; 52° 41' W; 700 m). The climate in the region, according to Köppen's classification, is Cfa in transition to Cfb. The data of rainfall and temperature during the study are presented in Figure 1.

The soil in the experimental area, according to the criteria of the Brazilian Soil Classification System - SiBCS (EMBRAPA, 2006), was classified as distroferric Red Latosol, with undulating relief and clayey texture. Its chemical attributes in the layer of 0-20 cm, through the Mehlich-1 extraction method, were: pH ($CaCl_2$) - 4.5; P - 10.3 mg dm^{-3}; K - 0.23 $cmol_c$ dm^{-3}; organic matter - 44.2 g dm^{-3}; Al^{3+} - 0.26 $cmol_c$ dm^{-3}; Ca - 3.91 $cmol_c$ dm^{-3}; Mg - 1.64 $cmol_c$ dm^{-3}; CEC - 11 $cmol_c$ dm^{-3}; V% - 54; H + Al - 4.96 $cmol_c$ dm^{-3} and SB - 5.78.

In the winter cultivation, prior to experiment installation, the area was cultivated by an intercropping of cover crops:

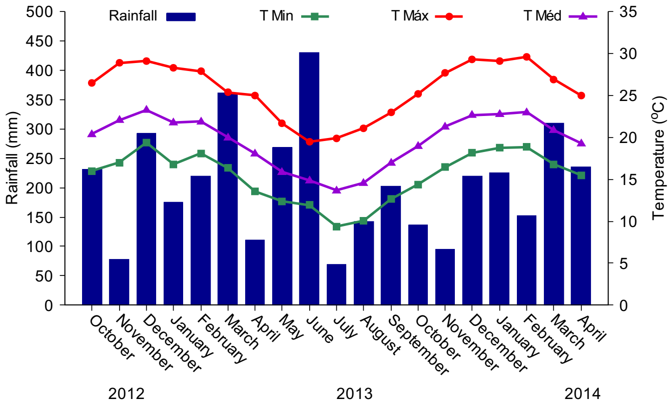

Figure 1. Cumulative rainfall and maximum, minimum and medium temperatures per month in the period from October 2012 to April 2014

common radish (*Raphanus sativus* L.), field pea (*Pisum arvense* L.), common vetch (*Vicia sativa* L.) and black oat (*Avena strigosa Schreb*.), sown on May 15, 2012. Cover crops were managed with a cutting roller on August 23, 2012, and desiccation on October 16, 2012, with the non-selective herbicide glyphosate [2.5 L of the commercial product (c.p.) ha^{-1}]. Between the maize crops of 2012/13 and 2013/14, black oat (cultivar Iapar 61) was sown broadcast in the cultivation area on June 19, 2013, and desiccated with glyphosate (2.5 L c.p. ha^{-1}) on October 30, 2013.

The experiment was set in randomized blocks with four replicates in two cultivation years, corresponding to the seasons of 2012/13 and 2013/14. The treatments consisted of inoculation of PGPB strains: control (without N and without inoculation); 30 kg of N ha^{-1} at sowing (N1); 160 kg of N ha^{-1} (30 kg ha^{-1} at sowing + 130 kg ha^{-1} as top-dressing); N1 + inoculation with *A. brasilense*, strain Ab-V5; N1 + inoculation with *A. brasilense*, strain HM053; N1 + inoculation with *Azospirillum* sp., strain L26; N1 + inoculation with *Azospirillum* sp., strain L27; N1 + inoculation with *Enhydrobacter* sp. strain 4331; N1 + inoculation with *Rhizobium* sp. strain 8121.

The single-cross maize hybrid 30F53 Herculex® was evaluated and its seeds were inoculated 12 h before sowing, at the proportion of 20 mL of the inoculant for each kg of seeds. The inoculants were prepared and provided by the Federal University of Paraná (UFPR), from a pure solution of bacteria at the concentration of 10^9 cells mL^{-1}. The strains HM053 and Ab-V5 are registered by the Ministry of Agriculture and the others were recently isolated from the roots of the maize variety 'Sol da Manhã', in Santo Antônio de Goiás-GO (L26 and L27) and from the roots of a double-cross hybrid (AG 2040) in Londrina-PR (4331 and 8121).

Sowing was performed using handheld maize planters (called "matracas") on October 25, 2012, and November 14, 2013. Each experimental unit consisted of six 5-m-long rows (0.8 m between rows) with five plants per linear meter. Fertilization was performed based on soil chemical analysis and on recommendations proposed by IAPAR (2003), through the application of 50 kg ha^{-1} of P_2O_5 and 50 kg ha^{-1} of K_2O in the sowing furrow, in both experiments. The fertilizers single superphosphate (18% of P_2O_5) and potassium chloride (58% of K_2O) were used as sources of P and K, respectively. After sowing, N fertilization was applied via broadcast, in the form of urea (46% of N) except for the control treatment. The rest of the N dose, for the treatment with 160 kg of N ha^{-1}, was applied as top-dressing in the V6 stage (Weismann, 2008). Phytosanitary tracts were performed according to the necessity and the recommendations for the crop.

Two plants were randomly collected in each plot for biometric evaluations in the vegetative (V8 - eight developed leaves) and reproductive (R1 - appearance of style-stigmas on the ear) stages. Before plant collection, basal stem diameter was measured at 5 cm from the soil surface and the canopy height was measured between the base and the highest point of the plant. Sampled plants were collected and dried in a forced-air oven at 65 ± 2 °C for 72 h, for the determination of shoot dry matter. Leaf area was determined by the method of dry matter of leaf discs, proposed by Benincasa (2003).

Harvest was manually performed on March 26, 2013, and on April 16, 2014, by collecting all ears from the evaluation area of the plot (two 3-m-long central rows). Grain yield was calculated based on the production of the evaluation area of each plot, in kg ha^{-1}, after correcting the moisture content to 13%.

The data were subjected to analysis of variance using the program GENES (Cruz, 2006) and means were compared by Tukey test at 0.05 probability level.

Results and Discussion

In the 2012/13 season, there was effect of inoculation on the variables plant height, in both development stages (V8 and R1), and stem diameter, in V8 (Table 1). However, in the 2013/14 season, the effect occurred on plant height (V8 and R1), stem diameter (V8), shoot dry matter (R1), leaf area (V8 and R1) and grain yield (Table 1).

In the 2012/13 season, for plant height in the V8 stage, the inoculation with the strain HM053 promoted increment of 33 cm (25%) in relation to the control, but without differing from the other treatments (Table 2). In the R1 stage, the inoculation with the strain HM053 and N fertilization of 160 kg ha^{-1} were superior to the control and to the N fertilization of 30 kg ha^{-1}, and similar to the other treatments (Table 2). However, in the 2013/14 season, all treatments, except N fertilization of 30 kg ha^{-1}, showed greater plant height compared with the control in V8 (Table 3). Such behavior occurred again in R1 and the control treatment showed lower plant height in comparison to the others, except for the treatment with 30 kg of N ha^{-1} and the inoculation with the strains Ab-V5 and HM053 (Table 3).

Plant height is a genetic characteristic influenced by the environment in which the plant develops and on which the availability of nutrients has great impact. Thus, the inoculation of PGPB may have promoted an effect similar to that of N fertilization, stimulating the development of plant roots and their potential of absorption of nutrients (Albuquerque et al., 2013).

As to the basal stem diameter in the V8 stage, in the 2012/13 season, the inoculation of the isolate HM053 surpassed the control treatment and the inoculation of the strain L27, without differing from the other treatments (Table 2). However, in

Table 1. Summary of the analysis of variance for the variables plant height (PH), basal stem diameter (SD), shoot dry matter (SDM), leaf area (LA), in the stages V8 and R1, and grain yield (YIELD) of hybrid maize (30F53) in the seasons of 2012/13 and 2013/14, as a function of seed inoculation with strains of growth-promoting bacteria

SV	DF	Mean square								
		PH V8	PH R1	SD V8	SD R1	SDM V8	SDM R1	LA V8	LA R1	YIELD
2012/13 season										
Block	3	381	32	10*	29**	683238	23351251	403	1320	1730203
Inoculation	8	365*	302**	8.2*	10	924498	15432240	1450	2392	2280039
Error	24	149	63	2.8	4.6	823125	14098812	621	1045	1061798
CV (%)		8.05	3.17	6.89	8.08	18.9	35.1	17.3	14.7	14.9
2013/14 season										
Block	3	216	128	2.6	6.8	536225	981071	618	17	1579459*
Inoculation	8	701**	404**	30**	6.4	855140	7811177*	1871*	1645*	2451614**
Error	24	142	119	6.0	5.7	687730	2404977	749	593	339537
CV (%)		7.74	4.24	8.50	11.2	22.1	15.9	19.5	16.9	17.9

SV - Source of variation; DF - Degrees of freedom; *, **Significant by F test at 0.05 and 0.01 probability level, respectively.

Table 2. Plant height (PH), basal stem diameter (SD), shoot dry matter (SDM) and leaf area (LA), in the stages V8 and R1, and grain yield (YIELD) of hybrid maize (30F53) in the 2012/13 season, as a function of seed inoculation with strains of growth-promoting bacteria

Treatment	V8 stage				R1 stage				YIELD kg ha^{-1}
	PH cm	SD mm	SDM kg ha^{-1}	LA dm^2	PH cm	SD mm	SDM kg ha^{-1}	LA dm^2	
Control	132 b	23 b	3,995	108	236 c	24	6,788	172	5,852
30 kg of N ha^{-1}	144 ab	25 ab	4,863	134	241 bc	26	9,906	199	6,102
160 kg of N ha^{-1}	157 ab	24 ab	4,804	155	260 a	29	13,291	241	7,199
A. brasilense (Ab-V5) + 30 kg of N ha^{-1}	157 ab	26 ab	5,087	159	256 ab	27	12,403	234	6,538
A. brasilense (HM053) + 30 kg of N ha^{-1}	165 a	27 a	5,687	174	260 a	28	11,020	232	7,871
Azospirillum sp. (L26) + 30 kg of N ha^{-1}	157 ab	25 ab	4,930	147	258 ab	28	10,219	251	7,718
Azospirillum sp. (L27) + 30 kg of N ha^{-1}	147 ab	23 b	4,262	130	248 abc	26	9,291	205	6,509
Enhydrobacter sp. (4331) + 30 kg of N ha^{-1}	154 ab	25 ab	4,661	142	254 abc	25	10,812	220	7,673
Rhizobium sp. (8121) + 30 kg of N ha^{-1}	154 ab	24 ab	4,850	148	255 ab	27	12,428	218	6,403
Mean	152	24.5	4,793	144	252	27	10,684	219	6,874

Means followed by different letters in the column differ significantly by Tukey test at 0.05 probability level

Table 3. Plant height (PH), basal stem diameter (SD), shoot dry matter (SDM) and leaf area (LA), in the stages V8 and R1, and grain yield (YIELD) of hybrid maize (30F53) in the 2013/14 season, as a function of seed inoculation with strains of growth-promoting bacteria

Treatment	V8 stage				R1 stage				YIELD kg ha^{-1}
	PH cm	SD mm	SDM kg ha^{-1}	LA dm^2	PH cm	SD mm	SDM kg ha^{-1}	LA dm^2	
Control	122 b	24 b	2,792	92 b	233 b	19	7,541 b	105 b	2,555 cd
30 kg of N ha^{-1}	147 ab	28 ab	3,225	122 ab	257 ab	21	8,741 ab	132 ab	2,840 cd
160 kg of N ha^{-1}	160 a	30 a	3,908	152 ab	268 a	22	11,105 ab	161 ab	4,536 a
A. brasilense (Ab-V5) + 30 kg of N ha^{-1}	163 a	32 a	4,235	151 ab	256 ab	21	9,643 ab	135 ab	3,229 bcd
A. brasilense (HM053) + 30 kg of N ha^{-1}	159 a	27 ab	3,859	146 ab	260 ab	19	8,242 b	128 ab	2,364 d
Azospirillum sp. (L26) + 30 kg of N ha^{-1}	164 a	32 a	3,980	144 ab	265 a	23	11,975 a	170 a	3,779 abc
Azospirillum sp. (L27) + 30 kg of N ha^{-1}	160 a	29 ab	3,757	145 ab	261 a	22	10,000 ab	151 ab	2,556 cd
Enhydrobacter sp. (4331) + 30 kg of N ha^{-1}	158 a	26 ab	3,721	145 ab	262 a	22	9,803 ab	153 ab	3,118 bcd
Rhizobium sp. (8121) + 30 kg of N ha^{-1}	156 a	32 a	4,187	167 a	261 a	22	10,517 ab	158 ab	4,267 ab
Mean	154	29	3,740	140	258	21	9,730	144	3,249

Means followed by different letters in the column differ significantly by Tukey test at 0.05 probability level

the 2013/14 season, the N fertilization of 160 kg ha^{-1} and the inoculation with the strains Ab-V5, L26 and *Rhizobium* sp. 8121 showed higher values of stem diameter in the V8 stage in comparison to the control treatment, but without differing from the others (Table 3). Ramos et al. (2010) observed that the inoculation of *Azospirillum* associated or not with N fertilization at sowing (30 kg ha^{-1}) surpassed the control treatment for collar diameter of maize plants. Increments in the development of maize stem diameter are directly related to the increments of the individual plant production, due to its function in the storage of soluble solids that may be used later, in the formation of grains (Fancelli & Dourado Neto, 2000).

The results of plant height and basal stem diameter, in general, show that the inoculation with PGPB, combined with 30 kg of N ha^{-1}, did not differ from the N fertilization of 160 kg ha^{-1}. According to Siqueira et al. (1999), when plants are colonized by *Azospirillum*, there is an increase in root hair density, rate of appearance of secondary roots and root surface, due to the production of phytohormones by the bacterium, with modifications not only in growth, but also in the morphology and density of root hairs. Such effect stimulates the absorption of water and nutrients from the soil, favoring the development of the plant as a whole.

For shoot dry matter and leaf area in the 2012/13 season, there was no effect of inoculation in both maize development stages (Table 2), while in the 2013/14 season shoot dry matter, in the R1 stage, was higher for the inoculation of the isolate L26 and lower in the control treatment and for the inoculation of the strain HM053 (Table 3). Still in the 2013/14 season, the highest values of leaf area, in comparison to the control treatment, were observed in the treatments with inoculation of *Rhizobium* sp. 8121 (V8) and the isolate L26 (R1); the other treatments showed intermediate and similar values of leaf area (Table 3).

The similar results between the inoculation of the strains associated with the N fertilizations of 30 kg ha^{-1} and 160 kg ha^{-1} reflect the effect of growth promotion caused by the PGPB, involving a set of mechanisms besides the production of phytohormones, such as biological control of phytopathogens, BNF and solubilization of phosphates (Bashan & Levanony, 1990; Pidello et al., 1993). In addition, the inoculation of PGPB associated with low N doses has shown greater efficiency for the plant-bacteria system, in comparison to isolated inoculation, while the application of high N doses reduces such efficiency due to the plant response to the stimulation in the absorption of the nutrient, limiting the biological process (Bárbaro et al., 2008).

For grain yield in the 2012/13 season, there was no difference between the treatments (Table 2). The lack of response of maize to the inoculation, as well as to the conventional top-dressing fertilization, may be associated with the favorable climatic conditions along the crop cycle (Figure 1) and the high yield in the control treatment. This high yield in the control treatment without N fertilization is due to the natural stock of N in the experimental area, since maize was cultivated after an intercropping of cover plants mainly composed of leguminous plants with low C/N ratio, increasing N availability in the system and its absorption by the plant (Bortolini et al., 2000).

In the 2013/14 season, the N fertilization of 160 kg ha^{-1} promoted mean yield of 4,536 kg ha^{-1}, surpassing the control treatment and those with N fertilization of 30 kg ha^{-1} and seed inoculation with the strains Ab-V5 and HM053 and with the isolates L27 and 4331. In this same year, the strain of *Rhizobium* sp. 8121 and the isolate L26 stood out with mean yields of 4,267 and 3,779 kg ha^{-1}, respectively, representing increments of 50 and 33% in relation to the isolated use of basal N fertilization (30 kg ha^{-1}), demonstrating the potential of the strain under these cultivation conditions.

It is also noticeable the difference in yield between the cultivation years; in the 2012/13 season, the mean maize yield was 6,874 kg ha^{-1} (Table 2), while in the 2013/14 season it was only 3,249 kg ha^{-1} (Table 3), representing a reduction of 53%. The expressive yield reduction in the 2013/14 season reflects the response of the plants to the abiotic stress suffered at the beginning of the development, despite the favorable climatic conditions (Figure 1), with the occurrence of storm with strong wind gusts between the stages V6 and V8, causing a random "lodging" of the plants. According to Weismann (2008), a decrease of 10 to 25% in yield may occur if the total distribution of leaves in the V8 stage is altered by the occurrence of biotic/abiotic factor. In addition, in the V8 stage, the plant finishes the definition of the number of grain rows on the ear (Weismann, 2008); therefore, it is more sensitive to stress in this period.

The results of the present study show that, despite being in the same cultivation area, the PGPB strains have different behaviors, due to abiotic variations. Thus, more studies on the behavior and selection of strains under different edaphoclimatic conditions must be conducted in order to consolidate the promising results that some strains showed in the present study. The possibility of combining strains that are efficient and show different behavior in the same inoculant may represent a viable alternative to potentiate the benefits of inoculation in maize.

Conclusion

The strain *Rhizobium* sp. 8121and the isolate *Azospirillum* sp. L26, associated with N fertilization of 30 kg ha^{-1}, promoted yields equivalent to that of N fertilization of 160 kg ha^{-1}, demonstrating great potential to be used in the inoculation of maize seeds.

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