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Initial performance of maize in response to NPK fertilization combined with *Herbaspirillum seropedicae*[1]

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ABSTRACT

The inoculation with plant growth-promoting bacteria can be a technological approach useful for increasing the production of maize. The objective of this study was to evaluate the initial performance of maize in response to application of doses of NPK combined with the inoculation of the diazotrophic bacteria *Herbaspirillum seropedicae* in an greenhouse experiment. The experiment consisted of six fertilizer levels: 0, 25, 50, 75, 100 and 200% of the recommended dose of NPK applied to maize inoculated and non-inoculated with *H. seropedicae*. At 30 days after the treatment application, the growth characteristics and nutritional status of the plants were evaluated. Plant development was influenced by fertilization, but it was enhanced by combination with the bacteria, which resulted in significant increases in the dry mass of shoots (7%) and leaf area (9%) when compared with non-inoculated plants. The results showed increases in the concentration of N (11%), P (30%) and K (17%) of maize plants in response to bacterial inoculation together with NPK compared with plants that were applied fertilize only. The greater consistency and stability response of the host plant to bacterization in the presence of chemical fertilizer indicate a promissory biotechnological approach for improving the initial growth and adaptation of maize to the cultivation environment.

Key words: *Zea mays* L., plant growth-promoting bacteria, nitrogen biological fixation, biofertilizer, biological feedstocks.

RESUMO

Desempenho inicial do milho, em resposta à adubação NPK combinada com a inoculação de *Herbaspirillum seropedicae*

A inoculação de bactérias promotoras de crescimento de plantas pode ser uma abordagem tecnológica útil para aumentar a produção de milho. O objetivo deste trabalho foi avaliar o desempenho inicial de plantas de milho, em resposta à aplicação de doses de NPK combinadas com a inoculação da bactéria diazotrófica *Herbaspirillum seropedicae*, em experimento em casa de vegetação. A matriz experimental consistiu em seis níveis de adubação: 0, 25, 50, 75, 100 e 200 % da dose de NPK recomendada, aplicados em plantas de milho inoculadas e não inoculadas com *H. seropedicae*. Aos 30 dias após a aplicação dos tratamentos, foram avaliadas as características de crescimento e nutricionais das plantas. O crescimento do milho foi influenciado pela adubação, mas foi reforçado pela combinação com a bactéria, que resultou em aumentos significativos na matéria seca da parte aérea (7 %) e área foliar (9 %), quando comparado com as das plantas não inoculadas. Os resultados indicaram incrementos no acúmulo de N (11 %), P (30 %)

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e K (17 %), em plantas de milho, em resposta à inoculação bacteriana em conjunto com NPK, quando comparado com os das plantas que apenas receberam adubação. A estabilidade e maior consistência da resposta da planta hospedeira para bacterização, na presença de fertilização química, apontam para uma ferramenta biotecnológica promissora para melhorar o crescimento inicial e adaptação de plantas de milho ao ambiente de cultivo.

Palavras-chave: *Zea mays* L., bactérias promotoras de crescimento de plantas, fixação biológica de nitrogênio, biofertilizantes, insumos biológicos.

INTRODUCTION

With the growing demand for increased yield in agriculture, also grows the demand for new technologies with less impact on natural resources. Among them, the use of micro-organisms promoters of plant growth may help or even meet the demand of nitrogen (N) and phosphorus (P) in different crops (Rodriguez & Fraga, 1999; Baldani et al., 2009; Baldotto et al., 2010).

N and P are present in limiting concentrations in tropical soils and affect agricultural yield. Mineral fertilizers are used to meet the need of these nutrients. Although effective, mineral fertilizers have shown growing increase in production costs, mainly because they often require non-renewable raw materials and/or demand high energy costs for their production (Novais & Mello, 2007).

Nitrogen exists in the soil system in many forms and changes (transforms) very easily from one form to another.

Because of nitrogen high mobility and transformations in the soil, the evaluation of its availability in the soil system is not commonly done by routine chemical analysis. Considering the important functions of this nutrient in the plant, such as protein synthesis, chlorophyll formation and alkaloid composition, among others, its low availability is synonymous with low yield. With respect to P, 90% of the analyses in the country show low levels of P available to crops, reaching less than 1 mg dm^{-3} by Mehlich-1 extractor. This low availability results in the high phosphate complexation on the surface of iron oxides (Fe) and aluminum (Al), which are predominant in the mineral fraction of tropical soils, in advanced stage of weathering, a factor that leads to applications of large amounts this nutrient in agriculture (Novais & Mello, 2007).

Considering the acreage and the production volume, the maize crop is a major activity for Brazil's GDP. The production of the crop was approximately 55 million tons in 2010/11 and the planted area was about 13 million hectares (Brazil, 2012). Maize is one of the main export products and has an important role in human and animal nutrition, with great economic and social importance. Moreover, it has growth potential because of the demand for energy of biofuels. However, in recent years this cereal acreage declined about 9%, due to the negative balance between production cost and market prices (Brazil, 2012). For this reason, technologies that reduce production costs must be developed, including those that reduce spending on nutrients.

One possibility to minimize production costs of maize and make it more competitive, is the use of microorganisms that fix atmospheric N (termed diazotrophs), in similar way that successfully occurred with soybean in Brazil (Hungria et al. , 2005).

The biological nitrogen fixation was innovative and allowed Brazil to become the second largest producer of soybean. The same result can also be obtained from crops of the Poaceae family that includes maize and sugar cane (Baldani et al., 2009). In these grasses, unlike pulses, there is no formation of nodules and these microorganisms colonize from the surface to the interior of the plant tissue (termed endophytes and epiphytic), respectively (Hallmann et al. 1997; Baldotto & Olivares, 2008).

For maize, the inoculation of the bacteria *Azospirillum* and *Herbaspirillum* brought positive results in the growth and development of plants (Baldani et al., 2009; Dotto et al., 2010; Pedrinho et al., 2010). However, there is little stability of the plant response to bacterization and the technology of diazotrophs needs to be adjusted to non-leguminous plants (Roesch, 2007).

In addition, there may be a continuum between forms of production, i.e., combinations of mineral nutrition and inoculation with growth promoting microorganisms to provide productions of maximum economic efficiency, or even the combined applications of these materials, providing technologies for more than one agriculture reality, eg, in terms of production costs and demand for the consumer market.

In this context, this study aimed to study the initial growth and mineral nutrition of maize (Zea *mays* L.) in response to application of NPK rates combined with the inoculation with the diazotrophic bacterium *Herbaspirilum seropedicae*.

MATERIAL AND METHODS

Treatments and experiment conditions

The experiment consisted of six fertilization levels: 0, 25, 50, 75, 100 and 200% of the recommended dose of NPK (De-Polli et al., 1988) applied to maize plants, inoculated and non-inoculated with endophytic diazotrophic bacteria (Table 1). The experiment was conducted in a greenhouse in a completely randomized design with four replications, totaling 48 experimental units.

The experimental units consisted of a plastic/polythene vase of 1 dm^3, filled with subsurface horizon soil of a cohesive dystrophic Oxisol, of a depth of 40-80 cm, in Campos-RJ. The soil showed the following characteristics: Clay content = 48%; pH = 4.3; MOS = 3.33 dag dm-^{3}; P = 1 mg dm^{-3}; K = 101 mg dm^{-3}; Ca^{2+} = 0.30 cmolc dm^{-3}; Mg^{2+} = cmolc 0.30 dm^{-3}; Al^{3+} = 0.80 cmolc dm^{-3}; H + Al = 3.20 cmolc dm^{-3}; Na = 0.10 cmolc dm^{-3}; SB = 0.96 cmolc dm^{-3}; t = 1.76 cmolc dm^{-3}; T = 4.16 cmolc dm^{-3}; V = 23% and m = 45%. Measurements followed the recommendations of Silva (2009).

Liming was performed 30 days before sowing, according to the recommended fertilization guidelines, using the criterion of aluminum neutralization and increased availability of Ca and Mg (Alvarez & Ribeiro, 1999). The soil of each experimental unit was placed in plastic bags, along with lime, homogenized and moistened to field capacity. After incubation, fertilization was carried out in each experimental unit. The 04-14-08 NPK fertilizer was homogenized with the soil in plastic bags at the rates shown in Table 1. Then, the soil of each treatment was added to a plastic container in which six were sowed maize seeds, and 5 days after emergence the seedlings were thinned, leaving two plants per pot.

The inoculum was obtained by growing *Herbaspirilum seropedicae* HIII 206 in DYGS liquid medium (Döbereiner et al. 1,995) under constant shaking at 120 rpm for 24 hours at 30 °C. The bacterial suspensions were adjusted for cell number of 108 cells mL^{-1}. The inoculation was performed ten days after seeding, by adding 3 mL of inoculum with an automatic pipette, on the surface of the soil and at the collar of the maize plants (genotype UENF 506-8) bearing three leaves.

Morphological analyses

At 30 days after germination, the plants were collected for measurement of the following variables: number of leaves (NL), plant height (ALT) measured by the distance of the plant collar up the sheath of the first fully expanded leaf, using a graduate ruller; length of the largest fully expanded leaf (CFE); stem diameter (DC), using a Starret 727 digital caliper; leaf area (LA), using a bench top LI-3100 leaf area meter (LICOR, USA); root fresh matter (MFR); shoot fresh matter (MFPA); root dry matter (MSR) and shoot dry matter (MSPA) in an forced air oven at 60 °C for 7 days. After weighing, the root dry matter and shoot dry matter ratio (RPA) was calculated.

The SPAD index, which estimates the amount of chlorophyll through the green color intensity was determined using a portable SPAD-502 (Minolta, Japan) chlorophyll meter, 30 days after germination.

Nutrient analysis

After drying, the maize shoots were ground in Wiley mill, with sieves of 0.25 mm (60 mesh). Then, the material was crushed, sieved, digested with sulfuric acid and

Table 1. Treatments combined with the factors under study

Treatments(1)	NPK(2)	NPK amount(3)		Lime amount(4)
	%	kg ha^{-1}	g/vase	g/vase
NPK0	0	0	0,00	1,36
NPK25	25	161	3,21	1,36
NPK50	50	321	6,43	1,36
NPK75	75	482	9,64	1,36
NPK100	100	643	12,86	1,36
NPK200	200	1.286	25,71	1,36
NPK0+B	0	0	0,00	1,36
NPK25+B	25	161	3,21	1,36
NPK50+B	50	321	6,43	1,36
NPK75+B	75	482	9,64	1,36
NPK100+B	100	643	12,86	1,36
NPK200+B	200	1.286	25,71	1,36

(1) Treatments: NPK 0, 25, 50, 75, 100 and 200% of the dose recommended for cultivation of maize (De-Polli et al. 1988); B = inoculation of *Herbaspirillum seropedicae*. (2) NPK = 04-14-08 formulation. (3) Quantity of NPK applied according to the fertilizer recommendation, considering requirement for phosphorus of 90 kg ha^{-1} of P_2O_5 by the chemical soil analysis in Table 2. For example: to apply 100% of the recommended dose, it was calculated 643 kg ha^{-1} of NPK 04-14-08 and considering a final population of 50,000 plants ha^{-1}, this dose was equivalent to 12.86 g/pot. (4) Amount of lime applied according to the liming requirement of 2.7 Mg ha^{-1}, according to Alvarez & Ribeiro (1999).

hydrogen peroxide, and total N, P, K, Ca and Mg were determined (Silva, 2009). N was determined by the method of Nessler. P was determined using molecular absorption spectrophotometer (colorimetry) at a wavelength of 725 nm. The determination of K was made by flame photometry. Ca and Mg were measured by atomic absorption spectrophotometry. The contents of N, P, K, Ca and Mg were calculated by multiplying the dry mass of shoots (kg) by the content (g kg^{-1}) of the nutrient considered.

Count of diazotrophs

Counting of bacteria was carried out using the Most Probable Number (MPN), described by Döbereiner et al. (1995). Samples of 1 g of root macerate were washed in 9 mL of saline solution and serial dilutions were made up to 10^{-7}. Then, a 0.1 mL aliquot of each dilution was added to vials containing 5 mL of semisolid JNFb medium. The flasks were incubated for seven days at 30 °C. The count of bacteria was estimated by referring to the table of McCrady, for three replicates per dilution. It was considered positive growth the formation of an aerotactic band on the surface of the medium.

Statistical analyzes

Results were subjected to analysis of variance and the effects of treatments for qualitative variable, were unfolded into mean contrasts (Alvarez & Alvarez, 2006). For the quantitative factors, regression equations between the means of the variables and levels of NPK with and without inoculation of bacteria (obtaining response curves) were adjusted. The application of the F test in the unfolding of the factors was performed at 1, 5 and 10% probability. In the regression analysis, the models were tested when having coefficient of determination greater than 0.65. The regression equations were used to determine the level of fertilizer in the absence and presence of bacterial inoculum, to achieve maximum physical efficiency of dry matter production of maize shoots, calculated from the derived regression equation to determine the maximum point of the response curve in both the absence and in the presence of bacteria. The rate values for maximum efficiency were substituted in the regression equations for each of the other variables in order to estimate them for this condition.

RESULTS

Growth of shoots

The analysis of growth of shoots indicated, in general, positive effect of treatments that combined NPK mineral fertilizer with the growth-promoting bacteria *Herbaspirillum seropedicae* compared with the use of only fertilizers. Table 2 shows that the bacterial inoculation with fertilizer resulted in plants with significant increments of 3, 4, 9, 7 and 9% in the variables height, length of leaf, mass of fresh matter of shoots, mass of dry matter of shoots and leaf area. The stem diameter was an exception, decreasing by 2% with the use of bacteria combined with chemical fertilizers.

Table 3 shows the regression equations, adjusted between the mean growth characteristics (dependent variables, y) of maize and levels of NPK in the absence and presence of inoculated bacteria (independent variable, x). Among the regression equations of Table 3, it is observed in the penultimate variable, the response curve of dry matter of shoots (MSPA), which was used to obtain the maximum rates of physical efficiency (MEF), i.e., rates that reflected higher mass accumulation in plants. For the unfolding of NPK and NPK + B, both MEF levels were 200% of the demand for fertilization, resulting in MSPA equal to 1.90 and 2.18 g per plant, respectively. It is worth noting also that when the variation in the growth of the treated plants showed a coefficient of determination R2 <0.65, the estimation was represented by the mean ($\hat{y} = \bar{y}$) rates of NPK combined or not with the application of bacterial growth promoters. Generally, the shape of the quadratic response was observed both in the presence and absence of bacterial inoculum combined with the mineral fertilizer.

Substituting the means of levels of NPK and NPK + B, on the condition of maximum physical efficiency of MSPA, in the regression equations of Table 3, we obtained the means of the other variables of growth of shoots. These means determined for the condition of maximum efficiency, were used to obtain the relative differences between the treatments NPK and NPK + B and the control treatment, using the equation: Relative Increments (%) = [(mean of treatment - mean of control)/mean of control] x 100.

All differences observed marked increases over the control. For variables NF, ALT, DC, DFE, MFPA, MSPA and AF, there were increments of 4 and 8%; 3 and 17%; 15 and 22%; 10 and 11%; 35 and 97%; 34 and 74% and of 35% and 85%, of the treatments NPK and NPK + B over the control, respectively.

It was found that the estimated relative increments were higher for treatments that received mineral fertilization together with bacterial inoculation (NPK + B) in comparison with the treatments that received only mineral fertilizer (NPK) in all studied variables.

Growth of the root system

The data of root growth was analyzed in a similar way to the data of growth of shoots and the means and the regression equations are shown in Tables 4 and 5, respectively. The results revealed that root growth varied according to the levels of NPK in both the absence and in the presence of inoculum, without, on average, significant contrast for the comparison with or without inoculation (B^- vs. B^+).

Table 5 also reveals a lack of adjustment of regression models between most variables of root growth and levels of NPK, i.e., there was little effect of mineral fertilizer levels on the plant root system. However, the relationship between root and shoot decreased 85%, with the application of increasing rates of NPK, combined or not with bacterization.

Nutritional status

On average, the contents of N, P and K (Table 6) increased with the application of bacteria together with

Table 2. Characteristics of growth of shoots of 30-old-day maize plants in response to fertilization with NPK combined or not with inoculation of the growth promoting bacteria *Herbaspirillum seropedicae*; mean contrast; relative increase (IR), mean square error (QMR) and coefficient of variation (CV)

Treatments [1]	**Characteristics of growth of shoot**[4]						
	NF	**ALT**	**CFE**	**DC**	**MFPA**	**MSPA**	**AF**
		cm		mm	g		cm^2
NPK0	5,75	45,75	42,10	5,17	8,58	1,25	286,67
NPK25	5,63	43,38	44,80	5,24	8,65	1,38	290,06
NPK50	5,50	40,25	41,70	4,99	7,83	1,20	291,56
NPK75	5,75	42,13	44,00	5,52	9,05	1,38	319,31
NPK100	6,13	50,88	45,70	5,18	10,68	1,58	342,74
NPK200	6,00	47,25	49,50	5,72	13,25	1,90	438,58
NPK0+B	6,00	45,00	45,10	4,70	7,93	1,25	280,88
NPK25+B	5,63	40,50	40,70	4,82	7,90	1,18	274,13
NPK50+B	5,75	43,38	45,70	5,25	10,23	1,45	345,97
NPK75+B	5,63	47,50	45,00	5,31	10,65	1,60	343,89
NPK100+B	6,00	49,00	47,70	5,22	10,68	1,60	358,60
NPK200+B	6,25	52,50	53,60	5,95	15,73	2,18	533,96
Contraste B^- *vs.* B^+ [2]	$0,08^{ns}$	1,38*	1,67*	-0,09*	0,85**	0,10**	28,08**
IR (%)[3]	1	3	4	2	9	7	9
QMR	0,13	31,43	22,49	0,18	4,12	0,08	3899,31
CV (%)	6,19	12,30	10,40	8,07	20,05	18,96	18,19

[1] Treatment: 0, 25, 50, 75, 100 and 200% of the recommended NPK dose; B = inoculation of *Herbaspirillum seropedicae*. [2] Contrast: B^- = no inoculation; B^+ = inoculation. [3] Relative increase: 100 (xy)/y, where x is the mean of the highest value and y the mean of the treatment with the lowest value. [4] Characteristics of growth of the shoot: NF = number of leaves; ALT = plant height (cm); DC = stem diameter (mm); CFE = fully expanded leaf length (cm); MFPA = fresh matter mass of shoots (g); MSPA = dry mass of shoots (g); AF = leaf area (cm2). ^{ns*} and ** = not significant at 10% and significant at 5% and 1% probability level by the F test, respectively.

Table 3. Regression equations of the characteristics growth of shoots of 30-day-old maize plants, in response to application of NPK doses combined, or not, with inoculation of the growth promoting bacteria *Herbaspirillum seropedicae*

Variable[1]	**Unfolding**	**Regression equations**	**R^2**
NF	NPK	$\hat{y} = \bar{y} = 5{,}80$	
	NPK + B	$\hat{y} = 5{,}866\text{-}0{,}003x+0{,}00003^{(P<0{,}16)}x^2$	0,656
ALT	NPK	$\hat{y} = \bar{y} = 44{,}95$	
	NPK + B	$\hat{y} = 42{,}470+0{,}049x + 0{,}00001^{**}x^2$	0,746
DC	NPK	$\hat{y} = \bar{y} = 5{,}30$	
	NPK + B	$\hat{y} = 4{,}724+0{,}007x\text{-}0{,}00001^{o}x^2$	0,924
CFE	NPK	$\hat{y} = 42{,}630+0{,}012x+0{,}0001^{*}x^2$	0,811
	NPK + B	$\hat{y} = 43{,}410+0{,}014x+0{,}0001^{*}x^2$	0,827
MFPA	NPK	$\hat{y} = 8{,}289+0{,}005x+0{,}0001^{*}x^2$	0,906
	NPK + B	$\hat{y} = 7{,}821+0{,}029x+0{,}00005^{**}x^2$	0,960
MSPA	NPK	$\hat{y} = 1{,}2497+0{,}0013x+0{,}00001^{*}x^2$	0,888
	NPK + B	$\hat{y} = 1{,}1946+0{,}0041x+0{,}000004^{**}x^2$	0,957
AF	NPK	$\hat{y} = 282{,}2+0{,}280x+0{,}002^{**}x^2$	0,988
	NPK + B	$\hat{y} = 278{,}0+0{,}614x+0{,}003^{**}x^2$	0,966

[1] Variable: NF = number of leaves; ALT = plant height (cm); DC = stem diameter (mm); CFE = fully expanded leaf length (cm); MFPA = fresh matter mass of shoots (g); MSPA = dry mass of shoots (g); AF = leaf area (cm2). **, *, $^{\circ}$ and ^{P} = significant at 1, 5, and 10% probability.

the fertilizer, with increments of 11, 30 and 17%, respectively, compared with treatments receiving only mineral fertilizer. For Ca, Mg, on the other hand, there was no effect of bacteria application. The SPAD index decreased by 4% with inoculation.

It was found that most of the variables showed significant increase by inoculating *Herbaspirillum seropedicae* together with NPK (Tables 6 and 7).

Substituting the values of the levels of NPK and NPK + B, in the physical condition of maximum efficiency of MSPA, in the regression equations of Table 7, provided the values of the nutritional content of maize plants, which, in turn, were used to estimate the relative increments compared with the control treatments. The increments of treatments containing NPK and NPK + B over the controls were 41 and 24% for N; 59 and 190% for P, 47% and 84% for K; 50 and 12% for Ca; 22 and 55% for Mg; 3 and 0 for SPAD, respectively.

Bacterial Count

We observed an increased number of diazotrophs in the roots of plants from seeds inoculated with *Herbaspirillum seropedicae* when compared with non-inoculated plants (Figure 1), confirming the effect of bacterization.

The most probable number (MPN) of bacteria showed that the fertilization interfered in the population of diazotrophs of the inoculated plants. The increasing levels of NPK caused an increase in bacteria inoculated and then a stabilization, followed by the fall in the population. Thus, the largest populations were found in plants inoculated that received 25 to 75% NPK (Figure 1).

Table 4. Characteristics of root growth of 30-day-old maize plants in response to the application of NPK doses combined or not with inoculation of the growth promoting bacteria *Herbaspirillum seropedicae*; mean contrast; relative increase (IR), mean square error (QMR) and coefficient of variation (CV))

Treatments [(1)]	**Characteristics of growth of root**[(4)]		
	MFR	**MSR**	**RPA**
		g	
NPK0	11,15	1,53	1,22
NPK25	11,23	1,10	0,80
NPK50	13,43	1,23	1,02
NPK75	14,08	1,13	0,82
NPK100	14,36	1,20	0,76
NPK200	13,73	1,25	0,66
NPK0+B	11,39	1,15	0,92
NPK25+B	12,19	1,10	0,94
NPK50+B	15,01	1,30	0,90
NPK75+B	12,73	1,08	0,67
NPK100+B	12,50	1,15	0,72
NPK200+B	13,97	1,30	0,60
Contraste B^- *vs.* B^+ [(2)]	-0,53^{ns}	-0,06^{ns}	-0,61^{ns}
IR (%)[(3)]	4	5	1
QMR	4,19	0,08	0,11
CV (%)	15,49	23,60	18,30

[(1)] Treatment: 0, 25, 50, 75, 100 and 200% of the recommended NPK dose; B = inoculation of *Herbaspirillum seropedicae*. [(2)] Contrast: B^- = no inoculation; B^+ = inoculation. [(3)] Relative increase: 100 (xy)/y, where x is the mean of the highest value and y the mean of the treatment with the lowest value. [(4)] Characteristics of root growth: MFR = root fresh matter; MSR = root dry matter; RPA = root/shoot ratio (dry matter). ^{ns*} = not significant at 10% probability level by the F test.

DISCUSSION

There inoculation of *Herbaspirillum seropedicae* together with NPK promoted growth of shoots and improved the mineral nutrition of maize plants as compared to applying only chemical fertilizers. The positive effects of inoculation with *H. seropedicae* on the host plant, although not yet fully elucidated, can be attributed to biological fixation of atmospheric nitrogen (Urquiaga et al., 1992), phosphate solubilization (Pedrinho et al., 2010), synthesis of phytohormones (Radwan et al., 2004) and production of ACC deaminase (Rothballer et al. 2008).

In this study, no significant differences were observed in dry matter accumulation in roots of maize in response to application of NPK rates combined or not with the inoculation of *H. seropedicae*. It was found that with increasing doses of fertilizers, there was a decrease in the root/shoot ratio, confirming the increased availability of nutrients in the root environment.

In studies of inoculation with bacteria that promote plant growth without application of fertilizers, root growth was due mainly to the increased emission of root hairs, promoted by auxin stimuli secreted by the bacteria (Dobbelaere et al. 1999; Radwan et al. 2004). In this work, *H. seropedicae* stimulated the accumulation of nutrients in the shoots of maize plants, probably because of the greater nutrient uptake by roots. Increments of 30% in P content, in the level of fertilization of maximum physical efficiency, with inoculation of *H. seropedicae*, for example, can be attributed to the stimulus of the root formation in the presence of bacteria, which would result in higher P uptake by root interception. Increasing the root surface would, also, result in a greater absorption of P arising from transport mechanisms, diffusion and mass flow (Novais & Mello, 2007). Although this hypothesis can not be confirmed in this study, the use of bacteria, acting as *H. seropedicae*, was able to improve the efficiency of absorption and accumulation of P. Since much of phosphatic fertilizers recommended from non-labile P (Mello & Novais, 2007), the bacterial inoculation could promote economic efficiency in the use of the fertilizer.

The increases in the contents of N and K in the plants inoculated with *H. seropedicae* can also be explained by the increased absorption of these nutrients in the root environment. Moreover, for N, the supposed positive effects of atmospheric nitrogen fixation by *H. seropedicae* are added up (Baldani et al., 2009; Dotto et al., 2010). Notably, the inoculation of *H. seropedicae* in maize increases yield and reduces the amount of nitrogen fertilizer required by the crop (Reis et al., 2009). However, the effect can be negative because it is dependent on the maize genotype (Dotto et al., 2010).

The MPN of diazotrophs showed that the fertilizer in small doses favors an increase in the bacterial population inoculated, and levels of 25 to 75% of NPK favored a greater permanence of inoculum in the root environment. The structural events involved in the interaction between *H. seropedicae*-host plant have been well described for different cultures (Baldotto et al., 2011) and can be summarized in the steps: adhesion, anchoring, infection and colonization. The initial events of the interaction are dependent on a rhizosphere environment favorable to bacterial growth, including various factors such as moisture and nutrient availability. In this study, for the best efficiency of bacterization, the ideal would be to use levels of up to 75% NPK.

The instability of the host plant response to inoculation with growth-promoting bacteria is an obstacle for the expansion and consolidation of the use

Table 5. Regression equations for the characteristics of root growth of 30-day-old maize plants in response to fertilization with NPK doses combined, or not, with inoculation of the growth promoting bacteria *Herbaspirillum seropedicae*

Variable(1)	Unfolding	Regression equations	R^2
MFR	NPK	$\hat{y} = \bar{y} = 13{,}50$	
	NPK + B	$\hat{y} = \bar{y} = 12{,}97$	
MSR	NPK	$\hat{y} = \bar{y} = 1{,}24$	
	NPK + B	$\hat{y} = \bar{y} = 1{,}18$	
RPA	NPK	$\hat{y} = 1{,}126-0{,}005x+0{,}00001^{o}x^2$	0,688
	NPK + B	$\hat{y} = 0{,}967-0{,}003x+0{,}000007^{o}x^2$	0,818

(1) Variable: MFR = root fresh matter (g); MSR = root dry matter (g); RPA = root/shoot ratio (dry matter). º = significant at 10% probability.

Table 6. Nutritional characteristics of 30-day-old maize plants in response to application of NPK doses combined with inoculation of the growth promoting bacteria *Herbaspirillum seropedicae*; mean contrast; relative increase (IR), mean square error (QMR) and coefficient variation (CV)

Treatments(1)	Nutritional characteristics(4)					
	N	P	K	Ca	Mg	SPAD
	mg/planta					
NPK0	9,57	1,83	8,85	0,15	0,28	17,25
NPK25	10,98	2,60	13,75	0,21	0,28	15,42
NPK50	10,16	2,35	8,91	0,17	0,23	16,22
NPK75	11,11	3,21	11,11	0,23	0,32	17,30
NPK100	15,69	4,33	13,14	0,25	0,37	15,15
NPK200	16,26	4,46	16,68	0,30	0,36	17,85
NPK0+B	11,28	2,64	11,17	0,19	0,27	14,83
NPK25+B	8,18	2,14	10,50	0,19	0,26	15,20
NPK50+B	15,37	3,20	11,87	0,25	0,34	17,33
NPK75+B	13,47	4,27	14,18	0,24	0,33	17,63
NPK100+B	10,96	4,62	16,80	0,25	0,35	15,33
NPK200+B	22,83	7,63	20,39	0,31	0,43	15,23
Contraste B^- *vs.* B^+ (2)	1,39**	0,95**	2,08**	0,02ns	0,02ns	-0,61**
IR (%)(3)	11	30	17	10	7	4
QMR	3,66	0,45	1,95	0,001	0,002	2,74
CV (%)	29,06	36,32	21,25	29,17	27,03	11,13

(1) Treatment: 0, 25, 50, 75, 100 and 200% of the recommended NPK dose; B = inoculation of *Herbaspirillum seropedicae*. (2) Contrast: B^- = no inoculation; B^+ = inoculation. (3) Relative increase: 100 (xy)/y, where x is the mean of the highest value and y the mean of the treatment with the lowest value. (4) Nutritional characteristics: content of nutrients: N, P, K, Ca, Mg = nitrogen, phosphorus, potassium, calcium, magnesium, respectively (mg/plant); SPAD. ns* and ** = not significant at 10% and significant at 5% and 1% probability level by the F test, respectively.

of this new technology (Baldani et al., 2009). To minimize this problem, alternatives such as adequacy of inoculation vehicles and the management of the rhizospheric environment should be sought (Bucher & Reis, 2008). Within this context, this study indicates the possibility of adequate rates of chemical fertilizers that provides increases in bacterial colonization and performance.

Table 7. Regression equations for the nutritional characteristics of 30-day-old maize plants in response to fertilization with NPK doses combined, or not, with inoculation of the growth promoting bacteria *Herbaspirillum seropedicae*

Variable[(1)]	Unfolding	Regression equations	R^2
N	NPK	$\hat{y} = 9{,}199 + 0{,}050\ x - 0{,}00007^{o}x^2$	0,782
	NPK + B	$\hat{y} = 11{,}00 - 0{,}005\ x + 0{,}0001^{o}x^2$	0,757
P	NPK	$\hat{y} = 1{,}728 + 0{,}027\ x - 0{,}00007^{*}x^2$	0,874
	NPK + B	$\hat{y} = 2{,}243 + 0{,}019\ x + 0{,}00004^{**}x^2$	0,965
K	NPK	$\hat{y} = \bar{y} = 12{,}07$	
	NPK + B	$\hat{y} = 10{,}11 + 0{,}056\ x - 0{,}00002\ x^2$	0,935
Ca	NPK	$\hat{y} = 0{,}157 + 0{,}001\ x - 0{,}000001^{*}x^2$	0,859
	NPK + B	$\hat{y} = 0{,}187 + 0{,}0001\ x - 0{,}000001^{**}x^2$	0,902
Mg	NPK	$\hat{y} = \bar{y} = 0{,}31$	
	NPK + B	$\hat{y} = 0{,}262 + 0{,}001x - 0{,}000001^{**}x^2$	0,911
SPAD	NPK	$\hat{y} = \bar{y} = 16{,}53$	
	NPK + B	$\hat{y} = \bar{y} = 15{,}92$	

(1) Variable: nutrient content: N, P, K, Ca, Mg = nitrogen, phosphorus, potassium, calcium, magnesium, respectively (mg/plant). **, *, $^{\circ}$ = significant at 1, 5 and 10% probability.

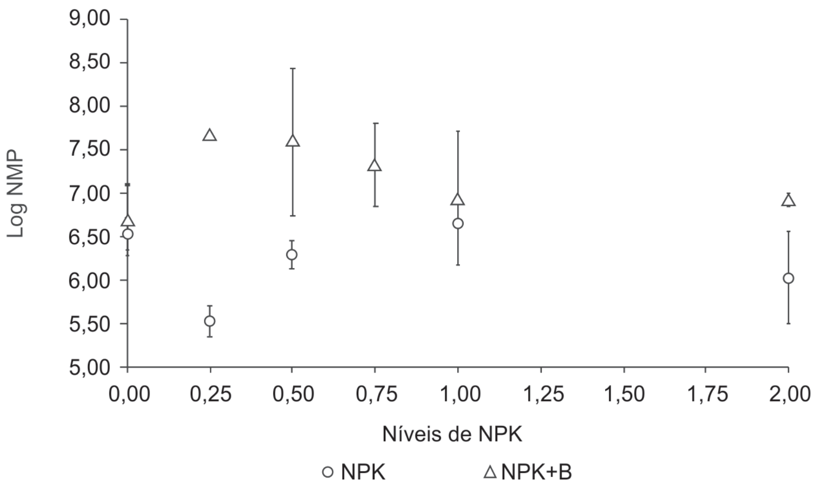

Figure 1. Bacterial populations (log NMP) present in maize roots 30 days after emergence, in response to the levels of NPK with or without inoculation of bacteria *Herbaspirillum seropedicae*.

CONCLUSIONS

The application of mineral fertilizer combined with inoculation with *Herbaspirillum seropedicae* resulted in greater increases in the characteristics growth of shoots and accumulation of N, P and K in shoots of maize plants when compared with the use of fertilizer only.

The inoculation of *Herbaspirillum seropedicae* did not influence the accumulation of dry matter of the root system of maize plants fertilized with NPK.

The root/shoot ratio decreased with the application of increasing rates of NPK.

The colonization of diazotrophs in inoculated roots of maize were enhanced with the application of fertilizer at rates between 25 and 75% of the NPK recommended dose.

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