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Improving sustainability of common bean production systems by co-inoculating rhizobia and azospirilla

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ABSTRACT

Common bean is likely the most important grain legume on population feeding for developing countries worldwide. With a natural ability to establish symbiosis with rhizobia, the inoculation technology is poorly exploited, especially in co-inoculation with others plant growth promoting rhizobacteria. This work aimed to determine the agronomical performance of common bean co-inoculated with *Rhizobium tropici* and *Azospirillum brasiliense*. A set of seven field experiments were carried out for three consecutive years in experimental areas and, majorly in commercial farms. Treatments consisted of non-inoculated control (NI), *N*-fertilizer treatment (NfT), single inoculation of *R. tropici* (Rt), *R. tropici* + one dose of *A. brasiliense* on seed (Rt+Ab1s), *R. tropici* + two doses of *A. brasiliense* on seed (Rt+Ab2s), *R. tropici* + two doses of *A. brasiliense* sprayed on plants (Rt+Ab2p) and *R. tropici* + three doses of *A. brasiliense* sprayed on plants (Rt+Ab3p). Evaluations were based on the nodule number (NN), nodule dry weight (NDW), root dry weight (RDW), shoot dry weight (MSPA), grain yield (GY), relative grain yield to NfT treatment (RGY:NfT) and relative grain yield to Rt treatment. The average of seven field experiments showed an increase of about 9%, 25%, 35% and 31% in NN, NDW, RDW and SDW, respectively as compared to Rt treatment. These increases over nodulation and plant growth resulted in a GY about 3200 kg ha^{-1}, representing an increase in GY of about 5% and 26% as compared to NfT and Rt treatments, respectively. The results indicate the feasibility of using rhizobia and azospirilla co-inoculation in commercial farms as an efficient technology in replacing *N*-fertilizers, helping to save expenses and preventing environmental risks.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) shows great social and economic importance worldwide (Builes et al., 2011), besides to contribute with about 25% of the protein on the dietary of the poorest Brazilian population (Hungria et al., 1997). The world production of the common bean is about 26.5 million tons lead by Myanmar, India, and Brazil. Each country contributes to this production with 17.5%, 15.5% and 12.4%, respectively (FAO, 2016). Due to its wide edaphoclimatic adaptation, in Brazil, common bean takes part of most production systems of small and medium farmers, been used for household consumption and income generation. Common bean is also present in high-performance production systems, characterized by the use of latest generation technologies and cultivation in dry season under irrigation.

Despite being a short-cycle plant, common bean requires high amounts of nutrients to achieve high yields in Cerrado soil conditions (Kluthcouski et al., 2000), a Savannah-like biome located in the Central region of Brazil. In particular, the N requirements by common bean are high. So, the maximum economic productivity of 2700 kg ha^{-1} under Cerrado conditions is achieved applying 167 kg ha^{-1} of N (Santos et al., 2003). Also, loses of this nutrient by exportation in seeds is about 50% (Fancelli and Dourado, 2007). Furthermore, the use of N-fertilizers increase the production costs and contributes to increased environmental risks, such as groundwater contamination with nitrite under irrigated production systems (Bortolotto et al., 2012) and the increase of greenhouse gas emission (Siqueira Neto et al., 2011).

Common bean can contribute to the reduction of the risks related to the use of N-fertilizers, since it can establish an

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association with *Rhizobium* to perform biological N_2 fixation (BNF). Many efforts have been applied to increase the adoption of BNF by common bean producers in Brazil; however, the agronomical efficiency of BNF under field experiments is still inconsistent. Studies have reported either high rates of productivity, ranging from 2500 to 3500 kg ha^{-1} (Mostasso et al., 2002; Pelegrin et al., 2009) to very low yields, from 600 to 1500 kg ha^{-1} (Raposeiras et al., 2006; Souza et al., 2011; Valadão et al., 2009). According to that, the complete replacement of N-fertilizers by inoculation is still a goal to be achieved, as compared to soybean crop, the most efficient model for BNF in the tropics. Thus, the need to improve the agronomic efficiency of BNF in the field is essential, and this challenge can be facilitated by the association of BNF with other growth promoting bacteria, like *Azospirillum*.

Co-inoculation of leguminous plants with symbiotic and associative bacteria produces synergistic effects that outweigh the results observed with single inoculation (Bárbaro et al., 2008). Besides BNF, the indole acetic acid may be involved in improving crop yields, since many genera are capable of producing this phytohormone such as *Rhizobium* spp. (Coatti et al., 2010; Schlindwein et al., 2008), *Azospirillum* spp. (Radwan et al., 2004; Reis Junior et al., 2004) and *Herbaspirillum* spp. (Radwan et al., 2004, 2005). Positive effects of this phytohormone in promoting common bean growth had already been reported (Hungria et al., 2013; Remans et al., 2008). All those bacteria can also promote plant growth by other mechanisms, such as: phosphate solubilization, salinity tolerance, biological control of phytopathogens and insects (Ahemad and Kibret, 2014).

In a study of common bean co-inoculated with *Rhizobium tropici* and *Azospirillum brasilense*, carried out in the South region of Brazil, the co-inoculation increased the grain yield by about 16% in comparison to the single inoculation with *Rhizobium tropici* (Hungria et al., 2013). However, there are no reports on the results of co-inoculation of common bean under Cerrado conditions, which shows very different soil condition as compared to the South region of Brazil. Thus, this work aimed to determine the effects of co-inoculation of *Rhizobium tropici* and *Azospirillum brasiliense* on the nodulation, growth and grain yield of common bean cropped in Cerrado soils.

2. Material and methods

2.1. Site description

Seven experiments were conducted with common bean in six different locations for three consecutive years (Table 1). The experiments were established in the Cerrado ecosystem on six municipalities of Goiás and Minas Gerais States, located in the Central region of Brazil (Table 1). According to Köppen's classification, the climate in all locations is classified as Aw. The rhizobial populations at each site were estimated using the most probable number technique (Vincent, 1970) and statistical tables with common bean plants cultivar Pérola. Rhizobial populations at each site are shown in Table 1.

2.2. Soil chemical and granulometry analysis

In each site, at the onset of experimentation, 20 soil subsamples (0–20 cm) were taken to evaluate soil chemical properties and soil granulometry. Chemical analysis followed basic procedures (EMBRAPA, 1997). Before being analyzed, soil samples were dried (60 °C for 48 h) and sieved (2-mm). Soil pH was determined in 0.01 M CaCl2 (1:2.5; soil/solution), after agitation for 1 h. Exchangeable Ca, Mg, and Al were determined in the extract obtained with 1 mol L^{-1} KCl (1:10; soil/solution) after agitation for 10 min. P and K contents were evaluated in the Mehlich-1 (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) extract (1:10; soil/solution) after agitation for 10 min. Aluminum was determined by titration with 0.015 Mo L^{-1} standardized NaOH, using bromothymol blue as indicator. Concentrations of Ca and Mg were determined in an atomic absorption spectrophotometer, K in a flame photometer and P by colorimetry, using the molybdenum-blue method and ascorbic acid as reducing agent. Organic matter was determined by Walkley & Black method. Soil characteristics before sowing at each site are shown in Table 2.

2.3. Inoculants, treatments and experimental design

According to Brazilian legislation, inoculants containing rhizobia must carry at least 10^9 cells g^{-1} or mL^{-1} of inoculant and, for *Azospirillum*-based inoculant at least 10^8 cells mL^{-1} of inoculant, since most *Azospirillum*-based inoculant in Brazil are in the liquid formulation. The technical recommendation for the common bean crop is that the dose of inoculant must supply 1.2 million cells $seed^{-1}$. In this study we used two commercial inoculants, MASTERFIX® feijão and MASTERFIX®L Gramíneas, containing *Rhizobium tropici* and *Azospirillum brasilense*, respectively. MASTERFIX® feijão contained the strain SEMIA 4070 (=CIAT899), while MASTERFIX®L Gramíneas contained the strains Ab-V5 and Ab-V6.

Every experiment included two controls, non-inoculated control (NI) and N-fertilizer treatment (NfT) without inoculation. On NfT a total of 80 kg N ha^{-1} was applied. Plots received 20 kg N ha^{-1} split-applied as urea at sowing and 60 kg N ha^{-1} split-applied at 25 days after emergence (DAE). Two doses (=2.4 million cells $seed^{-1}$) of inoculant were used in all treatments with inoculation of *Rhizobium tropici* (Rt). Finally, different concentrations of *Azospirillum brasilense* (Ab) were applied on seeds (s) or

Table 1
Locations, year, geographical coordinates, altitude to sea level, previous crop, sowing and harvest dates, and naturalized rhizobial populations at the sites where field experiments were performed.

Site/Year	Geographical coordinates		Altitude	Previous crop	Sowing	Harvest	Rhizobial cells[a]
	Latitude (S)	Longitude (W)	(m)				(CFU g^{-1} solo)
Itaberaí/2013	15°55′34.97″	49°44′07.57″	733	Maize	10/07/2013	04/10/2013	2.57×10^4
Goianésia/2014	15°14′42.95″	49°09′19.73″	829	Tomato	05/05/2014	06/08/2014	2.14×10^4
Santo Antônio de Goiás/2014	16°29′14.65″	49°17′01.27″	620	Maize	28/05/2014	21/08/2014	2.77×10^4
Unaí/2015	16°33′57.25″	47°08′20.29″	896	Maize	23/04/2015	03/08/2015	2.17×10^4
Cristalina/2015	16°52′42.45″	47°32′53.05″	1042	Soybean	29/04/2015	04/08/2015	4.35×10^4
Paracatu/2015	16°58′12.90″	46°18′54.99″	530	Maize	20/05/2015	20/08/2015	2.57×10^4
Santo Antônio de Goiás/2015	16°30′17.23″	49°17′16.85″	812	Rice	25/05/2015	26/08/2015	2.60×10^4

[a] Estimated by the most probable number (MPN) method (Vincent, 1970) using common bean as trap plants.