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Yield increase of soybean inoculated with a commercial arbuscular mycorrhizal inoculant in Brazil

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Arbuscular mycorrhizal fungi (AMF) play an important role in plant growth. However, there are no records of the use of AMF-based inoculants in agricultural crops in Brazil. The objective of this work was to evaluate the agronomic efficiency of a commercial inoculant containing the AMF *Rhizophagus intraradices* in combination with phosphate fertilization in soybean under different edaphoclimatic conditions in Brazil. Experiments were conducted in five states (Goiás, Mato Grosso, Minas Gerais, Paraná and Rio Grande do Sul) in a 2 x 3 factorial scheme, with two inoculation treatments (inoculated and non-inoculated seeds) and three doses of phosphate fertilization (0, 50 and 100% of the recommendation). At the end of the crop cycle (stages R2), it was found that the inoculant provided average increases of 29% in biomass (regardless of the applied P dose) and grain yield, and higher P uptake. It is concluded that the inoculant increases biomass production, P uptake and soybean yield under different edaphoclimatic conditions in Brazil, especially in soils that originally had low or medium levels of available P on the ground.

Key words: Arbuscular mycorrhizae, soybean, phosphate fertilization, *Rhizophagus intraradices*.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophic fungi that establish symbiotic relationships with plant roots (Smith and Read, 2008). The association provides benefits for plant growth and enables the AMF to complete their life cycle (Smith and Read, 2008). AMF absorb water and nutrients from the soil and transfer them to plants, while plants provide photoassimilates to fungi (Bi et al., 2005). The mycelium formed by the AMF outside the roots allows the plant to explore a larger volume of soil, and this extension of the root system increases the intake of water and nutrients (Smith and Read, 2008).

The symbiotic relationship between fungi and plants provides a number of benefits to surrounding plants and the environment (Jeffries et al., 2003; Berruti et al., 2016). Phosphorus (P) supply is the most important feature mediated by AMF (Smith and Smith, 2011). This benefit is ultimately observed primarily due to the low mobility of P in the soil (Smith and Read, 2008). AMF can contribute to plant growth through increased drought

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tolerance (Garg and Chandel, 2010), increased supply of other nutrients such as nitrogen, potassium and calcium (Sharif et al., 2011; Dania et al., 2013; Crespo, 2015). They also contribute to the formation of soil aggregates by the production and release of glycoproteins such as glomalin (Rillig, 2004). Arbuscular mycorrhizal fungi interfere with plant growth and soil biology the same way chemistry and physics influence the establishment of symbiosis between plants and AMF. The availability of P in soil is linked to the production of signals and responses between plants and AMF species, directly related to soil characteristics (Smith et al., 2011; Cely et al., 2016).

Around 300 species of AMF have been formally described (Glomeromycota Species List, 2019). Among those, *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler (until recently classified as *Glomus intraradices*) is a generalist species found distributed throughout different environments. The list of plant species benefited by AMF on growth and productivity include crops of agricultural importance such as soybeans (Spagnoletti and Lavado, 2015), corn (Guo et al., 2014), beans (Tajini et al., 2012), wheat (Ardakani et al., 2011) and cotton (Orak and Demir, 2011). Because of these characteristics, the use of that species for the formulation of commercial inoculants is recommended.

The production of AMF inoculants bumps into the obligatory symbiotic character of these fungi. This makes it difficult to develop an efficient inoculant production system that delivers large volumes of propagules in a short period. Traditional models of multiplication of these fungi use trap plants or root tissue culture (Vosátka et al., 2012; Berruti et al., 2015). Records of an efficient production process capable of supplying the Brazilian agricultural market with arbuscular mycorrhizal inoculants have not been found in the literature.

Farmers have increased their interest in sustainable practices, and this includes introducing, maintaining, and increasing AMF populations in production systems. The application of AMF in agriculture in annual crops, which represent large areas of agricultural production in Brazil (IBGE, 2019) should be studied. Thus, considering that the arbuscular mycorrhizal species *R. intraradices* has the potential to assure high yields in agricultural production systems with more sustainable management, studies are needed to prove these effects.

Thus, the objective of this work was to evaluate the agronomic efficiency of a commercial inoculant based on the AMF *R. intraradices*, in combination with different levels of phosphate fertilization, on soybean (*Glycine max* L.) growth and yield in five locations with different edaphoclimatic conditions in Brazil.

MATERIALS AND METHODS

Experiment locations

The experiments were conducted in the 2016/2017 crop season (September 2016 to March 2017), considering the soybean planting window, cultivars and driving practices of each tested site. For this, five (5) representative sites of soybean cultivated areas in the country (Figure 1) and with distinct edaphoclimatic characteristics were selected: Padre Bernardo in Goiás (GO), Ritápolis in Minas Gerais (MG), Tangará da Serra in Mato Grosso (MT), Pitangueiras in Paraná (PR), and Cachoeira do Sul in Rio Grande do Sul (RS) (Table 1).

Experimental specifications

Aiming to validate the efficiency of the AMF-based inoculant, the experiments were conducted in the field, following the protocols defined by MAPA (Brazilian Ministry of Agriculture, Livestock and Supply) for product registration based on the plant growth-promoting microorganism specifications, specifically the regulations contained in the IN SDA 13, from 03/25/2011 (Brasil, 2011) and the IN SDA 53, from 10/24/2013 (Brasil, 2013). Among the main requirements for the registration of microbial inoculants, we highlight the need to prove agronomic efficiency in at least four (4) regions with distinct edaphoclimatic characteristics, and that the role of the inoculant in reducing fertilization for the crop is proven.

All fertilization procedures, based on soil analysis and expected yield (SBCS, 2004), and crop treatments (Table 2) followed the recommendations for each site. The experiments were conducted until the complete pre-maturation cycle of the grains was completed, that is, in the R7 stage (Oliveira et al., 2016) (Table 1).

Experimental set-up

The experiments were implemented in subdivided plots, with mechanized sowing. In all cases, the experiments followed a 2 x 3 factorial scheme, with two inoculation levels (inoculated and non-inoculated seeds), three (3) levels of phosphate fertilization (no fertilization, 50%, and 100% of the recommendation for each site), and six replications (6), in subdivided blocks.

Every one of the 36 plots of each experiment had an area of 24 m^2 (4 m x 6 m), with a spacing of 1 m between plots. Each plot had a working area of 10 m^2, formed by the central portion of each plot (6 lines with a spacing of 0.5 m x 4 linear meters).

Inoculant characteristics

Rootella BR® (later registered under No. 22902 10000-0) was obtained by a mixed fungal propagule production system using trap plants (Berruti et al., 2015) and tissue culture (Diop, 2003; Srinivasan et al., 2014; Schuessler, 2015).

The inoculant was characterized and its purity certified, noting the number of propagules and the exclusive presence of propagules of the AMF species *R. intraradices*. This approach considered characteristics inherent to fungal spores, such as number, thickness, coloration, and ornamentation of the layers that cover the spore (https://invam.wvu.edu/). For inoculant quantification, the Most Probable Number (MPN) method (Oblinger and Koburger, 1975) was adopted to verify the concentration of 2,500 propagules per gram of product, as indicated on the label. The inoculant tested contained a mixed composition of spores and other fungal propagules (hyphae) and was formulated using sterile ultrafine vermiculite as an inert component.

Crop management

The seeds were inoculated with the initial addition of a liquid adhesive (Symbiosis Pró - MAPA - RS - 12834-1) and incorporation of the inoculant immediately before sowing. The recommended use

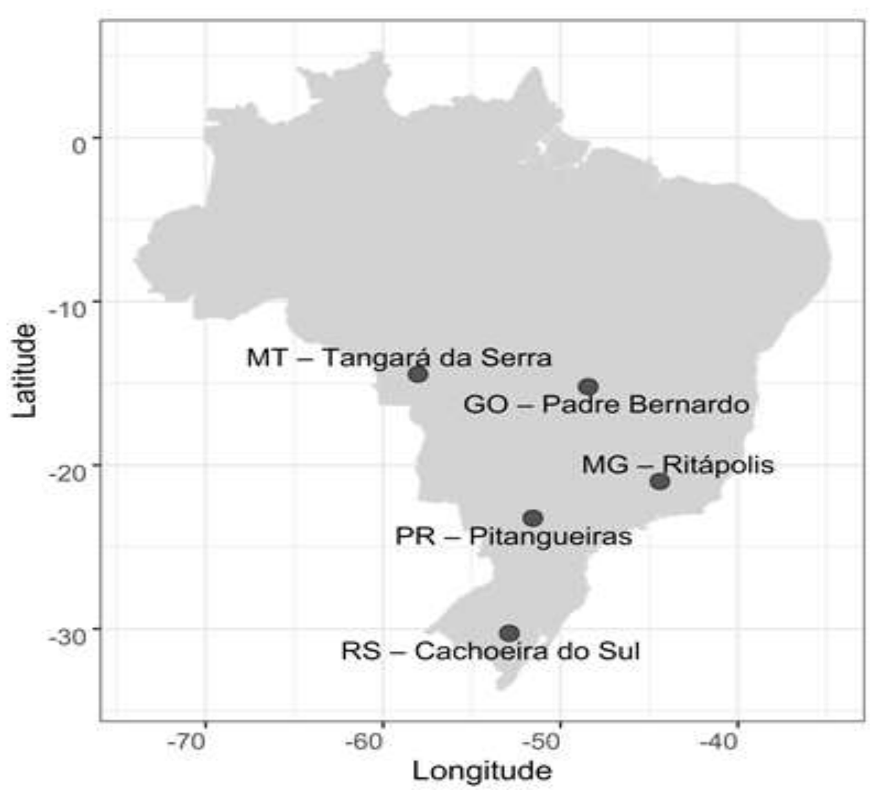

Figure 1. Locations of municipalities where experiments were conducted.

Table 1. Information on planting, harvesting, cultivar, soil characteristics, chemicals and agronomic practices employed in each location.

State/ municipality	Planting date (2016)	Harvesting date (2017)	Plant density (working area)#	Cultivar	Soil characteristics		
					P (mg/dm^3)†*	Clay (g kg^{-1})	N-P_2O_5-K_2O (kg ha^{-1})
GO – Padre Bernardo (15°12'29.9"S 48°26'28.6"W)	Nov 23	Mar 07	144	M8372 IPRO	6.71 (M)	600	0-80-40
MG – Ritápolis (20°59'39.1"S 44°24'15.4"W)	Oct 26	Mar 09	149	Nidera NS 7000 IPRO	1.10 (VL)	230	0-120-120
MT – Tangará da Serra (14°26'43.7"S 58°02'14.4"W)	Nov 07	Feb 15	288	TMG 2185 IPRO	4.80 (M)	680	0-120-120
PR – Pitangueiras (23°14'34.7"S 51°33'30.3"W)	Sep 21	Feb 27	224	DM 6563 IPRO	12.33 (H)	400	0-95-100
RS – Cachoeira do Sul (30°16'35.7"S 52°53'07.4"W)	Sep 04	Mar 01	151	Nidera 4823	16.00 (H)	260	0-60-140

† P determined by Mehlich 1 and the classes determined according to fertilizer recommendation system for each location; # Evaluations were based on the same number of plants per working area (30 plants); * Levels of P in the soil. M: Medium; VL: Very low; H: High.

Table 2. Edaphoclimatic characteristics and fungicides applied in each location.

State	Soil type	pH (H_2O)	Average temperature (°C)	Precipitation (mm/year)	Fungicides applied via seed	Fungicides applied during plant growth
GO	RL[1]	5.3	22.9	1431	Maxim XL[†]	Fox[‡] + Elatus*
MG	SCL[2]	5.3	19.2	1456	Maxim XL	No application
MT	RYL[3]	5.6	24.8	1830	Maxim XL	Fox + Cypress** + Priori Xtra[#]
RS	RU[4]	5.1	19.4	1692	Standak top (Estrobilurina)	Fox
PR	RL	5.9	19.0	1500	Standak top (Estrobilurina)	No application

[1] Red Latosol; [2]Sandy Clay Loam; [3]Red-yellow Latosol; [4]Red Udult. [†] Maxim XL: Fludioxonil + Metalaxyl−M; [‡] Fox: Trifloxystrobin + Protioconazol; *Elatus: Azoxystrobin **+** Benzovindiflupyr; **Cypress (Ciproconazole + difenoconazole); [#]Priori Xtra (Ciproconazole + azoxystrobin).

for the formulation tested was 1.0 kg of inoculant per hectare, regardless of the plant stand used. The plants were also inoculated via seed at the time of planting with nitrogen-fixing bacteria following the recommendations for soybean cultivation in the country.

Crop treatments performed in each area and region are described in Table 2. For one of the evaluated states (RS), seeds were treated with specific fungicides before planting. In addition to seed treatment, fungicides were also used throughout crop development in the state of RS, as specified in Table 2. The choice of products was based on the history of fungal diseases in each evaluated site. Weed and insect control was carried out using specific herbicides and insecticides for each situation.

Evaluations

Shoot dry biomass yield, P biomass content and accumulation, and grain yield was evaluated as required by MAPA IN SDA 13 and IN SDA 53. At the phenological stage R7 (Oliveira et al., 2016), 50 plants were collected from the four central lines of each plot (working area of 10 m^2). Drying of the biomass was performed in a forced air circulation oven at 60 °C to constant weight. Grain yield was determined from the yield obtained for 30 plants evaluated per plot at harvest time (considering the area mean stand), which were oven-dried following the same procedure described above.

The contents and accumulations of P in the tissues were determined from a sample composed of 20 newly mature leaves (without the petioles) harvested from the working area of each plot at the end of flowering (phenological stage R2) (Oliveira et al., 2016). Determinations followed Tedesco et al. (1995). Data was tested for homoscedasticity (Bartlet) ($p \leq 0.05$). Two-way analysis of variance was performed and Tukey's test was used for mean separation at 5 and 10% probabilities, as established by the legislation for registration of inoculants (Brasil, 2011, 2013). The analysis was performed using the software SISVAR v.5.3.

RESULTS

Table 3 shows the effects of mycorrhizal inoculation, phosphate fertilization and the interaction of these factors on biomass and grain yield, along with the levels and foliar accumulation of P. In general, the mycorrhizal inoculation and phosphate fertilization significantly influenced the afore-mentioned variables, from which the main effects are presented and discussed below.

There was an increase in biomass yield linked to the increase in phosphorus supplied by fertilization in three (3) of the five (5) sites tested. In Ritápolis (MG), a site with low soil available P, the response to soybean phosphate fertilization was clearer (Figure 2e).

Inoculation provided a 29% average increase in soybean aerial biomass yield, with a range of 17 to 41% increase. At all phosphate fertilization levels, the biomass of the inoculated plants was equal to or higher than that of the non-inoculated plants. Inoculation increased biomass yield in three (3) of the five (3) areas with 0% P (average 33% increase), in all areas in the treatment with 50% of the recommended P (average 32%), and in four (4) of the five (5) areas with 100% of the recommended phosphate fertilization (average 25%) (Figure 2).

The average P contents (Figure 3) in plant tissues at the five (5) sites were 1.13 and 1.70 $g.kg^{-1}$ in the non-inoculated and inoculated plants, respectively. The highest mean content was observed in MT (2.33 $g.kg^{-1}$) (Figure 3a), and the lowest value in GO (0.79 $g.kg^{-1}$) (Figure 3d), two sites with medium P content available in the soil. In four (4) of the five (5) areas there were positive responses of P content to inoculation, except in Pitangueiras, in the State of PR (Figure 3b). The average increase coming from the inoculation on the biomass P content compared to the non-inoculated plants was 72%. In MG and RS, sites with very low and very high levels of available soil P, respectively, positive responses to inoculation were seen, regardless of the applied P dose (Figure 3e and 3c), with average increases of 84% for MG and 190% for RS.

P accumulation (Figure 4) was higher in response to inoculation in all sites with no phosphate fertilization (average increase of 155%), in four (4) of the five (5) sites with 50% of the recommended dose (average increase of 297%), and in four (4) of the five (5) sites with 100% of the recommended dose of P (average increase of 92%).

Grain yield varied in response to AMF inoculation. Grain yield was on average 29% higher in the inoculated *versus* the non-inoculated plants, reaching 65% increases in some locations (Figure 5). In MT, the average yield increase of the inoculated plants was 28% (Figure 5a). It was 46% for PR (Figure 5b), 16% for RS (Figure 5c), 15% for GO (Figure 5d), and 40% for the

Table 3. F values and significance for the variables inoculation (I), phosphate fertilization (P) and IxP interaction on biomass and grain yield, as well as phosphorus levels and accumulations in soybean in different regions of Brazil.

Location	F value			
	Dry biomass	**Leaf P content**	**P accumulation**	**Grain yield**
Tangará da Serra-MT	I = 9.2** P = 4.7** IxP = 2.9*	I = 3.6^{ns} P = 2.7** IxP = 1.3^{ns}	I = 20.6** P = 7.4** IxP = 2.3^{ns}	I = 12.4** P = 5.5** IxP = 2.4^{ns}
Pitangueiras-PR	I = 72.6** P = 7.0** IxP = 2.2^{ns}	I = 0.3^{ns} P = 0.9^{ns} IxP = 1.2^{ns}	I = 27.2** P = 4.5** IxP = 0.7^{ns}	I = 80.3** P = 11.9** IxP = 2.9*
Cachoeira do Sul-RS	I = 42.9** P = 12.2** IxP = 2.4^{ns}	I = 538.7** P = 5.3** IxP = 5.1**	I = 97.8** P = 3.5** IxP = 4.8**	I = 13.9** P = 9.4** IxP = 1.6^{ns}
Padre Bernardo-GO	I = 19.7** P = 2.4^{ns} IxP = 1.1^{ns}	I = 4.8* P = 2.9* IxP = 2.1^{ns}	I = 17.1** P = 5.4** IxP = 0.7^{ns}	I = 6.3* P = 1.2^{ns} IxP = 0.4^{ns}
Ritápolis-MG	I = 22.1** P = 176.3** IxP = 3.4*	I = 41.8** P = 7.3** IxP = 0.9^{ns}	I = 73.3** P = 53.2** IxP = 6.3**	I = 7.5** P = 74.4** IxP = 2.4^{ns}

I=Inoculation; P=Phosphate fertilization; IxP= inoculation and phosphate interaction; **, * Significant effect at 5 and 10% probability; ns = non-significant effect.

state of MG (Figure 5e). Inoculation provided grain yield increases at two (2) of the five (5) sites where no phosphate fertilization was applied (average increase of 34%), in three (3) of the five (5) areas with 50% of the recommended dose of P (average increase of 35%), and in four (4) of the five (5) areas with 100% of the recommended P (average increase of 22%) (Figure 5). Grain yield responded to different rates of P in three of the tested areas, with higher intensity in Ritápolis (MG) (Figure 5e), the site with the lowest initial P content available in the soil.

Comparing the average yields of the three P levels with the non-inoculated treatment at 100% of the recommended P dose, inoculation provided grain yield increases of up to 29% (Figure 5). Grain yield of the inoculated plants was equal to or greater than the non-inoculated plants that received 100% of the P dose recommended. This was true to almost all sites and P dose treatments, the only exception being the 0% P inoculated treatment in MG, a site originally with low P available in the soil.

DISCUSSION

In most situations, there was a positive response to the application of the tested AMF inoculant. The literature provides substantial data on the beneficial effects of *R. intraradices*. This species is considered generalist, not only being associated with soybeans, but also with many other plant species of agronomic interest. Among the species that are known to benefit from interaction with *R. intraradices* are maize, beans, wheat, rice, oats and barley (Guo et al., 2014; Spagnoletti and Lavado, 2015; Tajini et al., 2012; Ardakani et al., 2011). Species of the genus *Rhizophagus* (formerly classified as *Glomus*) are cosmopolitan, and are present practically worldwide (Davison et al., 2015), and like other microorganisms such as *Rhizobium* and *Bradyrhizobium*, generally bring remarkable benefits when associated with plant species.

The effects of AMF on plant association may vary with the environmental conditions to which plants are exposed, such as higher or lower P availability in the soil. As observed in the present study (Figure 2), Cely et al. (2016), in a study conducted in the State of Paraná, showed that plants fertilized and inoculated with *Rhizophagus clarus*, a physiological species very close to *R. intraradices*, showed biomass increases of 24% when compared to fertilized and non-inoculated plants. This increase is very similar to that observed in the present work, with an average of 25% in fertilized (100% P) and inoculated plants. Thus, it can be stated that the inoculation promoted higher biomass yield, due to the greater efficiency of the inoculant, approaching the yields

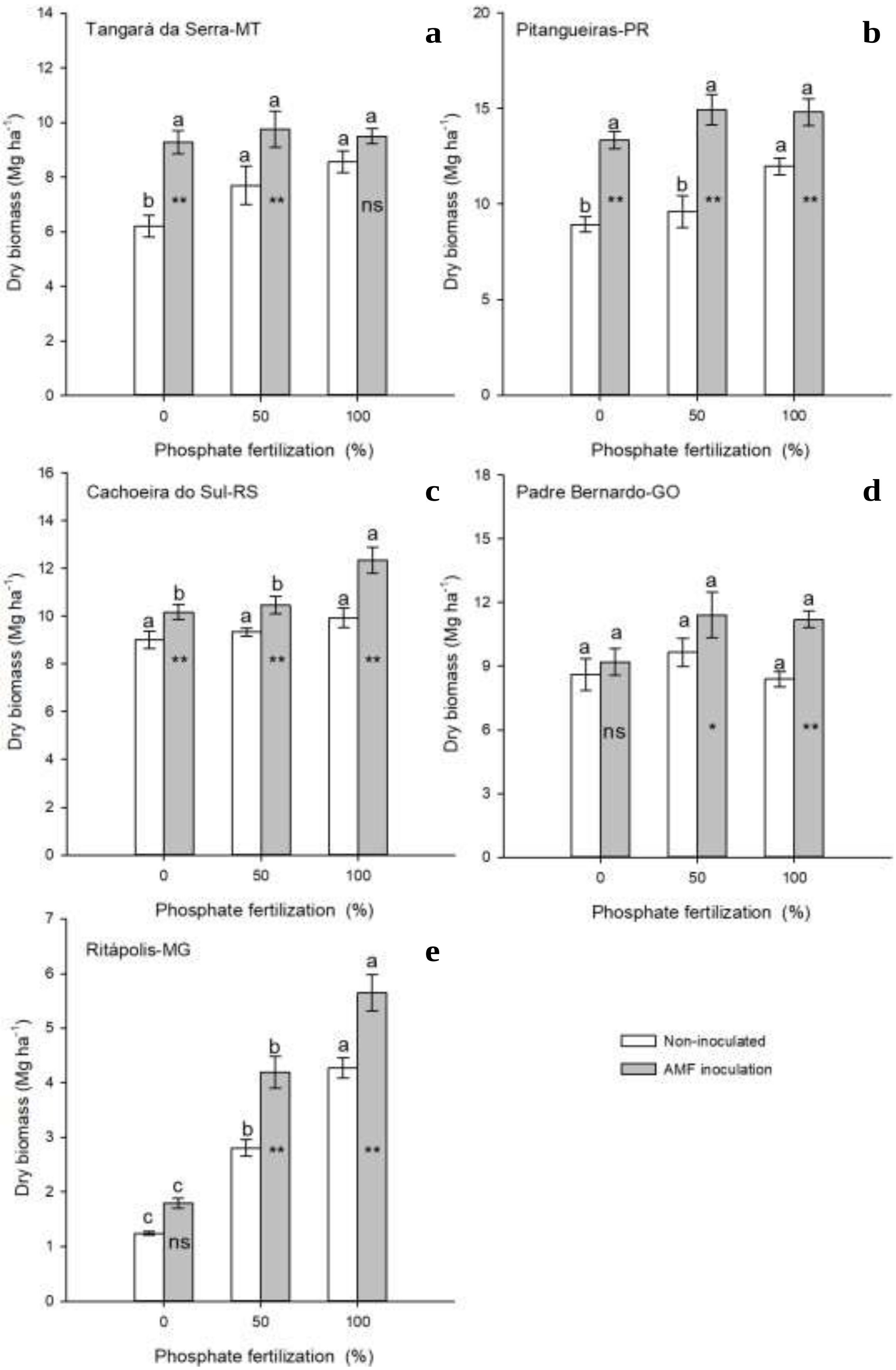

Figure 2. Effect of mycorrhizal inoculant application and phosphate fertilization on dry biomass of soybean (Mg ha^{-1}). **, * Significant effect of inoculation at 5 and 10% probability by F test within P doses; ns = non-significant effect of inoculation. Vertical bars represent the standard error. Averages followed by the same letter within the same inoculation treatment do not differ by Tukey's test at 5%/10% probability.

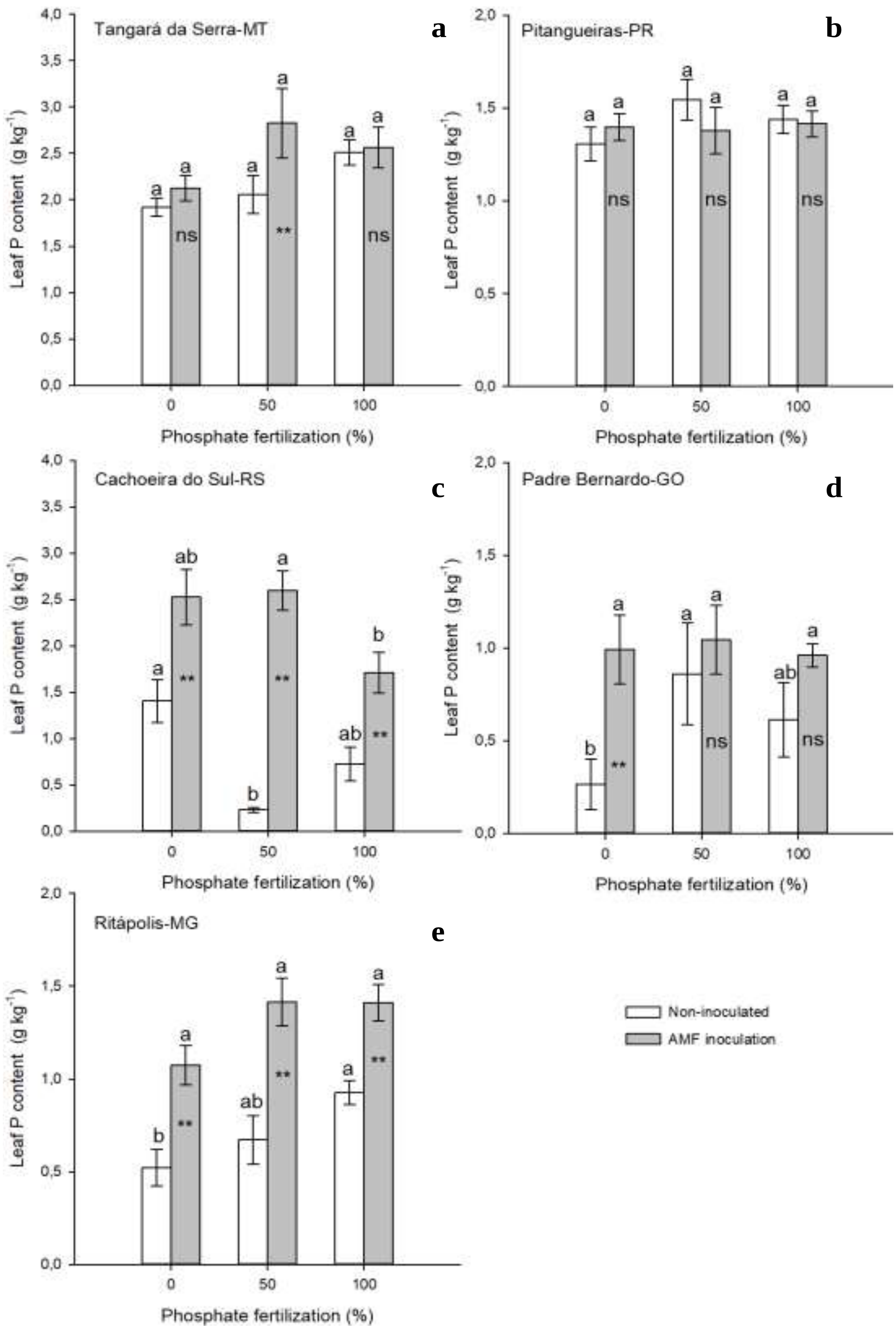

Figure 3. Effect of mycorrhizal inoculant application and phosphate fertilization on the level (g kg^{-1}) of foliar P on soybean. ** Significant effect of inoculation at 5% probability by F test within P doses; ns = non-significant effect of inoculation. Vertical bars represent the standard error. Averages followed by the same letter within the same inoculation treatment do not differ by Tukey's test at 5%/10% probability.

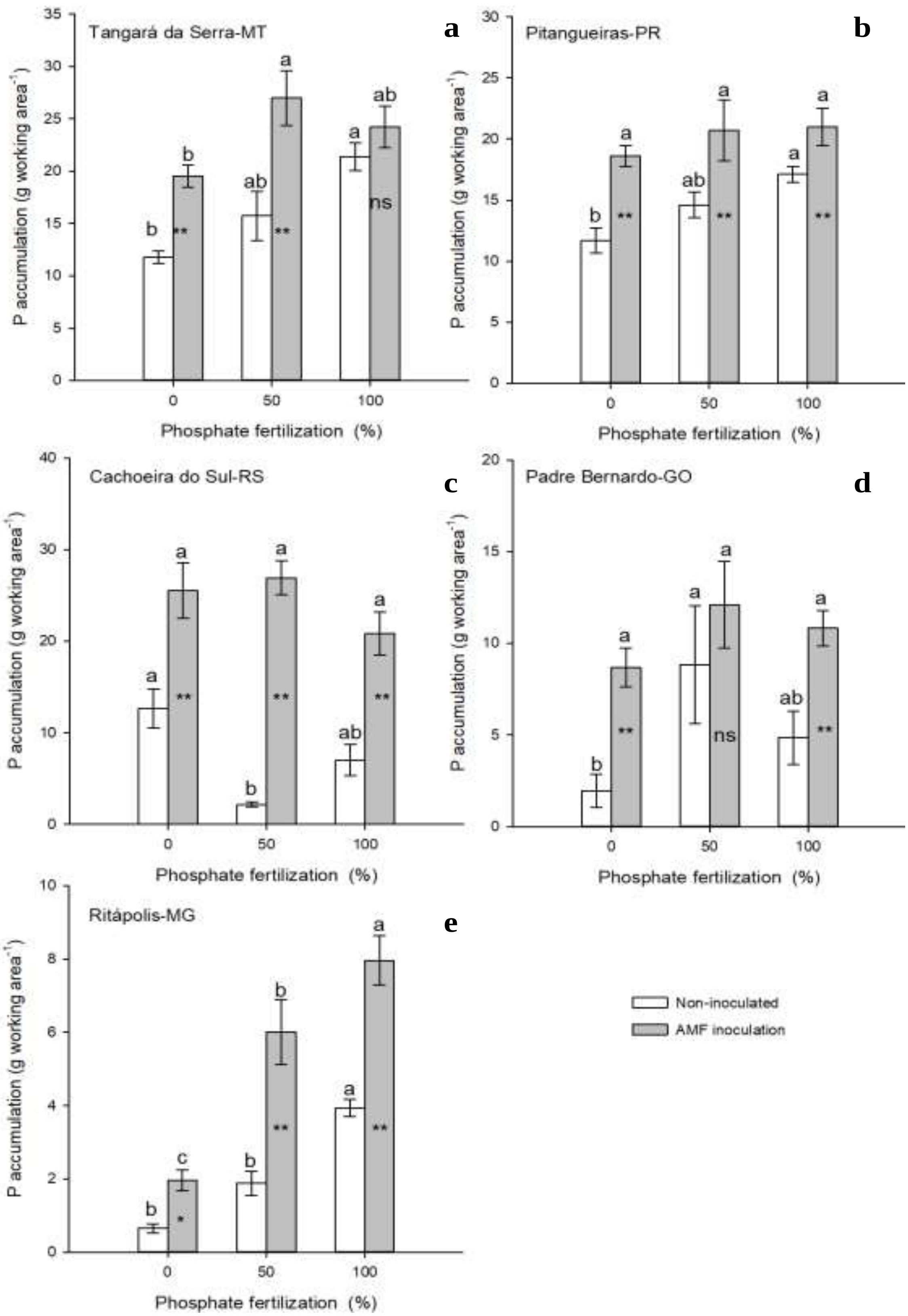

Figure 4. Effect of mycorrhizal inoculant application and phosphate fertilization on the accumulation (g working area^{-1}) of foliar P on soybean. **, * Significant effect of inoculation at 5 and 10% probability by F test within P doses; ns = non-significant effect of inoculation. Vertical bars represent the standard error. Averages followed by the same letter within the same inoculation treatment do not differ by Tukey's test at 5%/10% probability.

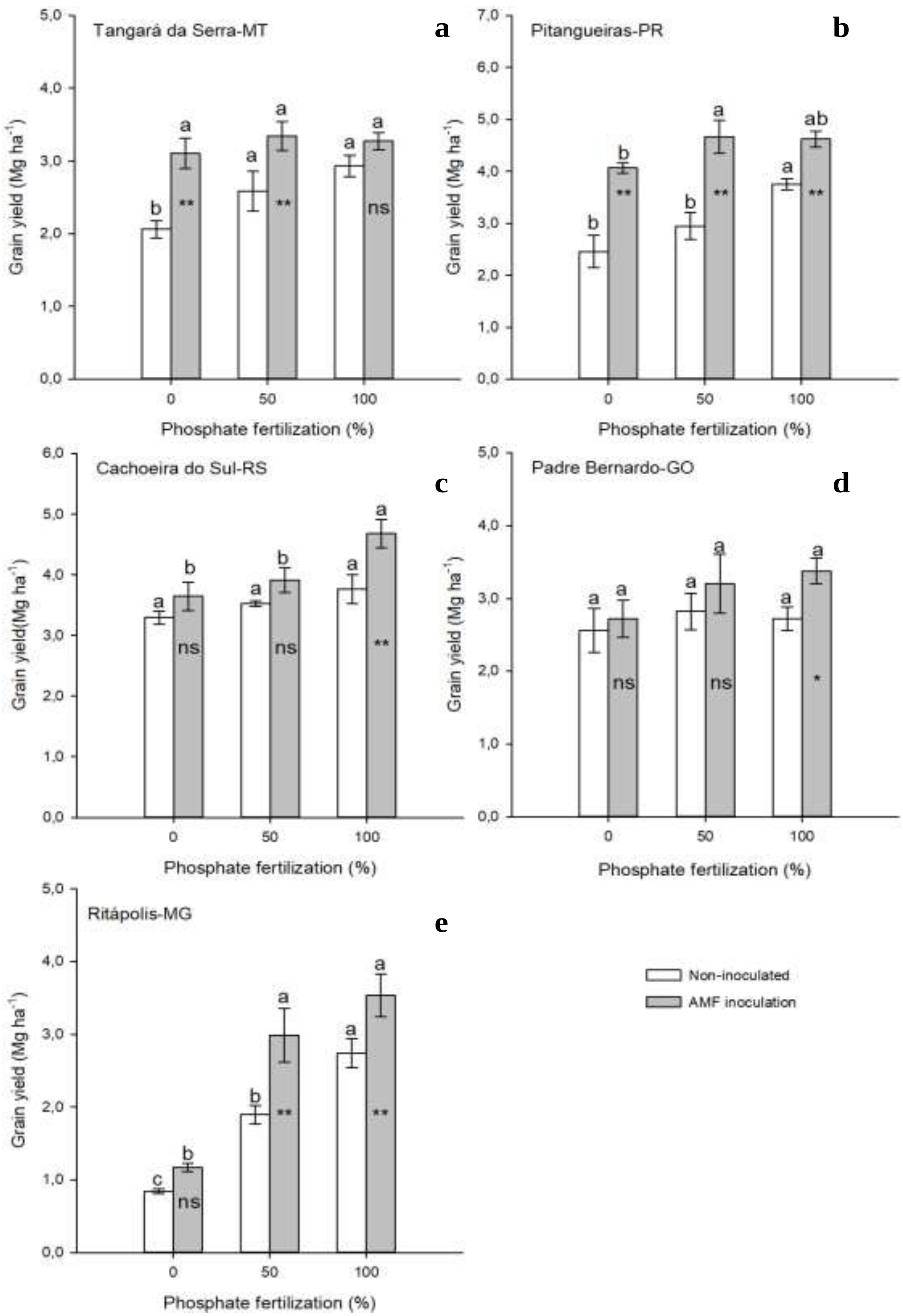

Figure 5. Effect of mycorrhizal inoculant application and phosphate fertilization on grain yield of soybean (Mg ha^{-1}). **, * Significant effect of inoculation at 5 and 10% probability by F test within P doses; ns = non-significant effect of inoculation. Vertical bars represent the standard error. Averages followed by the same letter within the same inoculation treatment do not differ by Tukey's test at 5%/10% probability.

observed for grain.

For normal plant growth during the growing season, Marschner (1995) shows that the P content should be between 3.0 and 5.0 g P kg^{-1} in the tissues, being toxic when exceeding 10 g P kg^{-1}. Some studies with soybean varieties have observed average levels that fall within this range (Corrêa et al., 2004; Broch and Ranno, 2012). However, in the present work, the average levels did not exceed 2.33 g of P kg^{-1} in the dry biomass. Rezende et al. (2009) also observed values lower than those described by Marschner (1995), with minimum and maximum contents of 0.97 to 1.23 g kg^{-1} of P. Thus, it is believed that although there are tables in the literature describing ranges for P values for soybean, these values may vary depending on the cultivation conditions and soybean cultivars evaluated.

In MG, a site with low available P, the effect of the inoculation on P content was clear (Figure 3e), supplying the P demand under low availability conditions. However, in places with higher P availability, such as in the State of RS, there seems to be a stimulus for extra P consumption. Even in the plots that did not receive phosphate fertilization, the P content is higher than in the control treatment (non-inoculated with 100% of P). The scientific literature highlights the potential of plant association with AMF in soils with different P concentrations available on plant nutrition and development (Harley, 1989; Bolan, 1991; George et al., 1995; Harrison, 1995; Bago et al., 2002; Ohtomo and Saito, 2005; Hodge et al., 2010; Smith et al., 2011; Cely et al., 2016). Thus, it can be seen that plants established in soils with high P content may respond differently to AMF inoculation compared to plants from low soil P available.

The largest increases in P accumulation in biomass were in RS (Figure 4c). This may have been caused by concentration as a function of higher P contents, since biomass yield did not follow these same trends. In addition, there appears to be a close relationship between AMF inoculation, biomass yield and P accumulation, especially in locations with less P availability in the soil, as there was a higher P accumulation in the five areas tested when little P was available in the soil (Figure 4).

Grain yield is the most important variable for farmers, and yield increases may be a result of different benefits provided by the association with AMF (Rilling et al., 2002; Ryan and Graham, 2002; Ruiz-Lozano, 2003; Hart and Forsythe, 2012).

Higher biomass yield, stimulated or not by inoculant application, seems to be related to higher grain yield. In the present work, grain yield of soybean plants responded to phosphate fertilization (Figure 5). The highest grain yields were observed in the places with high natural P availability in the soil: 3.8 and 3.74 Mg ha^{-1} in RS and PR (Figure 5b and c), respectively, followed by MT and GO with 2.88 and 2.90 Mg ha^{-1} (Figure 5a and d), respectively, and lastly MG with 2.2 Mg ha^{-1} (Figure 5e), a site with very low natural P availability. However, in addition to responding to P doses, grain yield was stimulated by inoculation, and the average increase of inoculated plants over non-inoculated plants, in the five sites tested, was 29% (ranging from 15 to 46%) (Figure 5). This result proves the efficiency of the *R. intraradices* mycorrhizal inoculant for increasing grain yield for soybean.

These results corroborate other studies in which two (Meghvansi and Mahna, 2009) and four (Meghvansi et al., 2008) soybean cultivars were employed. The authors verified increases in seed weight when plants were inoculated with *R. intraradices*, proving once again the economic potential of using AMF-based inoculants for this crop. Considerations on other benefits provided by AMF in soybean were made by Porcel et al. (2003), who claim that inoculation with AMF mitigates, for example, the effect of water deficiencies on this crop.

Responses of AMF inoculation are dependent on crop type, area history and interrelationships between living communities and environmental factors. The results obtained here show that P-based controlled fertilization and the addition of *R. intraradices*-based inoculant resulted in a significant increase in grain yield for soybean. In addition to the direct effect on growth and yield, AMF may allow reductions in P rates applied to the soil, since it was observed that with 50% of the P dose, inoculated plants had the same grain yield as non-inoculated plants with 100% of the recommended P. Thus, this reduction has a direct effect on soil quality, reducing the dependence on phosphate fertilizers, as well as reducing the expenses related to the purchase of fertilizers by the farmer.

Conclusion

The *R. intraradices* mycorrhizal inoculant increased the biomass yield, P uptake and soybean yield under different edaphoclimatic conditions, with more marked effects on soils that originally had low or medium levels of available soil P. On average, inoculation provided an increase of 29% in biomass (regardless of the applied P dose) and grain yield, and higher P uptake. Grain yield of the inoculated plants was equal to or even greater than the non-inoculated plants that received 100% of the recommended P. This result suggests a better use of farmer's monetary resources, as well as indicates that the use of environmentally sound approaches, such as biological inoculation of plants, cannot only reduce farms inputs of fertilizers, but also contributes towards more sustainable farming.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Ardakani MR, Mazaheri D, Mafakheri S, Moghaddam A (2011). Absorption efficiency of N, P, K through triple inoculation of wheat (*Triticum aestivum* L.) by *Azospirillum brasilense, Streptomyces* sp., *Glomus intraradices* and manure application. Physiology and Molecular Biology of Plants 17:181-192.

Bago B, Pfeffer PE, Zipfel W, Lammers P, Shachar-Hill Y (2002). Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. Metabolism and transport in AM fungi. Plant and Soil 244:189-197.

Berruti A, Lumini E, Balestrini R, Bianciotto V (2016). Arbuscular Mycorrhizal Fungi as natural biofertilizers: let's benefit from past successes. Frontiers in Microbiology 6:1-13.

Bi YL, Wu FY, Wu YK (2005). Application of arbuscular mycorrhizal in ecological restoration of areas affected by coal mining in China. Acta Ecologica Sinica 25:2068-2073.

Bolan NS (1991). A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134:189-207.

Brasil (2011). Ministério da agricultura, pecuária e abastecimento. Instrução normativa sda nº 13, de 24 de março de 2011. Altera a Instrução Normativa SARC no 05, de 6 de agosto de 2004. Diário Oficial da União: seção 1, sexta-feira, 25 de março de 2011.

Brasil (2013). Ministro de estado da agricultura, pecuária e abastecimento. Instrução normativa nº 53, de 23 de outubro de 2013. Que estabelece critérios de registro e produção de inoculantes. Diário Oficial da União: seção 1, 24 de Outubro de 2013.

Broch DL, Ranno SK (2012). Fertilidade do solo, Adubação e Nutrição da Cultura da Soja. In: Gitti e Roscoe Fundação MS, Tecnologia de Produção: Soja e Milho 2011/2012. Maracaju: Fundação MS. pp. 2-38.

Cely MVT, Oliveira AG, Freitas VF, Luca MB, Barazetti AR, Santos IMO, Gionco B Garcia GV, Prete CEC, Andrade G (2016). Inoculant of Arbuscular Mycorrhizal Fungi (*Rhizophagus clarus*) increase yield of soybean and cotton under field conditions. Frontiers in Microbiology 7:720.

Corrêa JC, Mauad M, Rosolem CA (2004). Fósforo no solo e desenvolvimento de soja influenciados pela adubação fosfatada e cobertura vegetal. Pesquisa Agropecuária Brasileira 39:1231-1237.

Crespo RJ (2015). Impact of arbuscular mycorrhizal fungi on the physiology of maize genotypes under variable nitrogen and phosphorus levels. Ph.D. Dissertation. University of Nebraska 150 p.

Dania SO, Fagbola O, Dania MI (2013). Response of Maize-Pigeon Pea intercrop to Arbuscular Mycorrhizal Fungi in nutrient depleted soil. Annual Review and Research in Biology 3:232-245.

Davison J, Moora M, Öpik M, Adholeya A, Ainsaar L, Bâ A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T, Johnson NC, Kane A, Koorem K,. Kochar M, Ndiaye C, Pärtel M, Reier Ü, Saks Ü, Singh R, Vasar M, Zobel M (2015). Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349:970-973.

Diop TH (2003). In vitro culture of arbuscular mycorrhizal fungi: advances and future prospects. African Journal of Biotechnology 2:692-697.

Garg N, Chandel S (2010). Arbuscular mycorrhizal networks: process and functions, a review. Agronomy for Sustainable Development 30:581-599.

George E, Marschner H, Jakobsen I (1995). Role of Arbuscular Mycorrhizal Fungi in uptake of Phosphorus and Nitrogen from soil. Critical Reviews in Biotechnology 15:257-270.

Glomeromycota Species List (2019). [cited 2019 Nov 15]. Available from: http://www.amf-phylogeny.com/amphylo_species.html.

Guo W, Zhao R, Fu R, Bi N, Wang L, Zhao W, Guo J, Zhang J (2014). Contribution of arbuscular mycorrhizal fungi to the development of maize (*Zea mays* L.) grown in three types of coal mine spoils. Environmental Science Pollution Research 21:3592-3603.

Harley JL (1989). The significance of mycorrhizal. Mycological Research 92:129-139.

Harrison MJ (1995). Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. Annual Review of Plant Physiology and Plant Molecular Biology 50:361-389.

Hart MM, Forsythe JA (2012). Using arbuscular mycorrhizal fungi to improve the nutrient quality of crops; nutritional benefits in addition to phosphorus. Science and Horticulture 148:206-214.

Hodge A, Helgason T, Fitter AH (2010). Nutritional ecology of arbuscular mycorrhizal fungi. Fungal Ecology 3:267-273.

IBGE (2019). Levantamento Sistemático da Produção Agrícola. Tabela 1 - Área, Produção e Rendimento Médio - Confronto das Estimativas Setembro/Outubro – Brasil. [cited 2019 Nov 15]. Available from: https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9201-levantamento-sistematico-da-producao-agricola.html?=&t=resultados.

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biology and Fertility of Soils 37:1-16.

Marschner H (1995). Functions of Mineral Nutrients: Macronutrients. pp. 229-314. In: Mineral nutrition of Higher Plants. Academic Press. 889 p. ISBN: 9780124735439.

Meghvansi MK, Mahna SK (2009). Evaluating the symbiotic potential of *Glomus intraradices* and *Bradyrhizobium japonicum* in Vertisol with two soybean cultivars. American-Eurasian Journal of Agronomy 2:21-25.

Meghvansi MK, Prasad K, Harwani D, Mahna SK (2008). Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. European Journal of Soil Biology 44:316-323.

Oblinger JL, Koburger JA (1975). Understanding and Teaching the Most Probable Number Technique. Journal of Milk and Food Technology 38:540-545.

Ohtomo R, Saito M (2005). Polyphosphate dynamics in mycorrhizal roots during colonization of an arbuscular mycorrhizal fungus. New Phytologist 167:571-578.

Oliveira Jr A, Castro C, Pereira LR, Domingos CS (2016). Estádios fenológicos e marcha de absorção de nutrientes da soja. Embrapa Soja.

Orak AB, Demir S (2011). Effects of Arbuscular Mycorrhizal Fungus and Different Phosphorus Doses Against Cotton Wilt Caused by *Verticillium dahliae* Kleb. Plant Pathology Journal 10:108-114.

Porcel R, Barea JM, Ruiz-Lozano JM (2003). Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytology 157:135-143.

Rezende PM, Carvalho ER, Santos JP, Andrade MJB, Alcantara HP (2009). Enxofre aplicado via foliar na cultura da soja [*Glycine max* (L.) Merrill]. Ciência e Agrotecnologia, 33:1255-1259.

Rillig MC (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. Canadian Journal of Soil Science 84:355-363.

Rilling MC, Wright SF, Eviner VT (2002). The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing.

effects of five plant species. Plant and Soil 238:325-333.

Ruiz-Lozano JM (2003). Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309-317.

Ryan MH, Graham JH (2002). Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant and Soil Sciences 94:263-271.

Schuessler A (2015). System and methods for continuous propagation and mass production of arbuscular mycorrhizal fungi in liquid culture. Patent WO 2015028601 A1.

Sharif M, Ahmad E, Sarir MS, Muhammad D, Shafi M, Bakht J (2011). Response of different crops to Arbuscular Mycorrhiza Fungal inoculation in phosphorus-deficient soil. Communications in Soil Science and Plant Analysis 42:2299-2309.

Smith SE, Jakobsen I, Gronlund M, Smith FA (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiology 156:1050-1057.

Smith SE, Read DJ (2008). Mycorrhizal Symbiosis, 3rd Edn. London: Academic. 2008.

Smith SE, Smith FA (2011). Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth New Paradigms from Cellular to Ecosystem Scales. Annual Review of Plant Biology 62:227-250.

Sociedade Brasileira de Ciência do Solo (SBCS) (2004). Comissão de Química e Fertilidade do Solo – RS/SC. Manual de adubação e de calagem para os estados do Rio Grande do Sul e de Santa Catarina. 10a. ed. Porto Alegre.

Spagnoletti F, Lavado RS (2015). The Arbuscular Mycorrhiza *Rhizophagus intraradices* Reduces the Negative Effects of Arsenic on Soybean Plants. Agronomy 5:188-199.

Srinivasan M, Kumar K, Kumutha K, Marimuthu P (2014). Establishing monoxenic culture of arbuscular mycorrhizal fungus *Glomus intraradices* through root organ culture. Journal of Applied and Natural Science 6:290-293.

Tajini F, Trabelsi M, Drevon JJ (2012). Combined inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.). Saudi Journal of Biological Sciences 19:157-163.

Tedesco MJ, Gianello C, Bissani CA (1995). Análise de solos, plantas e outros materiais. Boletim Técnico de Solos. 5. 2. ed. Porto Alegre: UFRGS, 174 p.

Vosátka M, Látr A, Gianinazzi S, Albrechtová J (2012). Development of arbuscular mycorrhizal biotechnology and industry: current achievements and bottlenecks. Symbiosis 58:29-37.