Chapter 14
Effect of Inoculation with Glomeromycota Fungi and Fertilization on Maize Yield in Acid Soils

Eliecer Cabrales, Danilo Lopez-Hernández, and Marcia Toro

Abstract The use of arbuscular mycorrhizae (AM) to improve crop productions in acid soils has been implemented through the production of inoculants based on native Glomeromycota fungi.The inoculants were isolated by wet sieving and decanting, sucrose gradient followed by reproducetion in trap pots with *Brachiaria decumbens*. Predominant species were: *Cetraspora pellucida*, *Scutellospora calospora*, *Ambispora leptoticha* and *Acaulospora mellea*. Each native species was reproduced individually followed by mixing the fungi together as a consortium. Inocula were applied to hybrid maize HIMECA 3003 and evaluated with 0, 27, 54 and 80 kg P_2O_5 ha^{-1} as diammonium phosphate (DAP). Seeds were sowed in 4.5 m × 3.6 m plots, in a randomized block design with factorial arrangement, for a total of 72 plots. Eight g of inoculum per sowing site were added (with 150 spores /100 g soil). The seeds were placed directly over the inoculum. Maize was harvested after 3 months to determine N and P in stem. The most efficient combination of P for N nutrition was 54 kg $P_2O_5.ha^{-1}$ and mixed inoculums. The highest P content in plant was obtained with 54 kg P_2O_5 ha^{-1} and *C. pellucida.* Fertilization combined with AM allowed 25% reduction of P fertilization and increased up to 100% in maize yield (from 2 to 4 t ha^{-1}), compared to yield data of the zone.

Keywords Arbuscular mycorrhizae (AM) · Acid soils · Maize · Fertilization

E. Cabrales
Facultad de Ciencias Agrícolas, Universidad de Córdoba, Montería, Colombia

D. Lopez-Hernández · M. Toro (✉)
Laboratorio de Estudios Ambientales, Instituto de Zoología y Ecología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela
e-mail: marcia.toro@ciens.ucv.ve

D. Zúñiga-Dávila et al. (eds.), *Microbial Probiotics for Agricultural Systems*, Sustainability in Plant and Crop Protection,
https://doi.org/10.1007/978-3-030-17597-9_14

14.1 Introduction

Tropical savannas are characterized by the presence of low fertility soils and predominance of the herbaceous component (Sarmiento et al. 2004). Nearly 270 million hectares of tropical savannas cover South America. Venezuela is the second country for extension of this type of ecosystems (Gómez and Paolini 2011). A significant portion of savanna soils is used for agricultural purposes, especially for maize without irrigation, with yields of approximately 2000 kg ha^{-1} (Vielma et al. 2005). However, agricultural activity can lead to the use of huge dose of fertilizers, producing an environmental contamination. The use of beneficial microorganisms (growth promoting bacteria and/or arbuscular mycorrhizae, AM) with complementary fertilization, may help to improve crops productivity. Glomeromycota fungi associate with 80% of vascular plants (Schüßler et al. 2001; Smith and Read 2008), thus forming arbuscular mycorrhizae. Maize is one of the crops that are frequently inoculated with AM (Harrier and Watson 2003; Rakshit and Bhadoria 2010). We focused on its study due to its importance as an important food source in the country.

Low P content of tropical soils favors the presence of AM (Cardoso and Kuyper 2006; Carrenho et al. 2007). Previous research have shown that reducing P fertilization (applied as rock phosphate) and inoculating AM, promoted maize yields of up to 3.1 Mg ha^{-1} (Hernández et al. 2012). Toro (2007) and Toro et al. (2008), report that several genera of Glomeromycota such as *Acaulospora*, *Glomus* and *Scutellospora* are associated to maize in Venezuela savannas soils. The aim of this study was to evaluate the efficiency of four species of native *Glomeromycota*, testing an inoculum composed of all native species as consortia, on maize growth and productivity in a field experiment carrid out using acid savanna soils.

14.2 Materials and Methods

This study was carried out at Estación Experimental La Iguana, in the southeastern area of Venezuela, Santa María de Ipire (8° 25’ N;65° 25′ E). Yellow maize HIMECA 3005 seeds were sowed at a 0.9 m distance between rows and 0.4 m between plants. Two seeds per site were sowed, with density of 55.000 plants ha^{-1}. Four species of Glomeromycota reported as native of these soils by Toro (2007) were used: *Cetraspora pellucida*, *Scutellospora calospora, Ambispora leptoticha*, *Acaulospora mellea* alone or as a consortium (all species). Inoculation was performed adding 8 g of each inoculum per site· The inoculum consisted of a mixture of spores, rootlets and mycelium of the corresponding fungi alone or as a consortium. Maize seeds were placed above the inoculum, to allow fungal colonization of roots. All inocula contained 150 spores · 100 g^{-1} of soil. Inoculation treatments were as follows: M0 = No AM inoculation; M1 = *C. pellucida*; M2 = *S. calospora*; M3 = *A. leptoticha*; M4 = *A. mellea* and M5 = consortium of all species.

Table 14.1 Chemical analysis of soil

	pH	O.M.	S	P	Ca	Mg	Na	K	Al	CEC	Cu	Fe	Mn	Zn	Mo
Depth 0–20 cm	1:1	%	($mg.kg^{-1}$)		($cmol.kg^{-1}$)						($mg.kg^{-1}$)				
	5.05	0.51	26.8	10.4	0.5	1.0	0.13	0.05	0.4	2.08	0.4	15.2	8.4	0.8	0.04

Fertilization was based on soil chemical analysis (Table 14.1) and crop requirements. The N dose was fractionated after 18-28-35 days of plant emergence by applying 45-53-52 kg/ha of N, as urea. Phosphorus and potassium doses were fully applied when sowing. P doses were: 0-27-54-80 kg/ha of P_2O_5 as DAP. 80 kg K ha^{-1} were applied as KCI.

P and N content in leaves were evaluated in the leaf opposite to the maize cob. Dried and ground foliar tissues were digested with binary mixture (sulfuric acid: perchloric acid, 4:1). P was determined as described by Murphy and Riley (1962) and N was determined by distillation with Kjeldahl (IGAC 2006).

We evaluated the effect of a combination of 4 doses of P and 6 inoculation treatments with the fungi species in foliar N and P content and grain yields. A randomized block design with factorial arrangement was used with the following factors: factor A, dose of P applied (4 levels or fertilization doses with P: 0-27-54-80 kg P_2O_5/ha); factor B, inoculation with fungi, with 6 levels (M0, M1, M2, M3, M4 and M5). Each experimental plot was integrated by 5 rows of maize. Sampling was carried out in three sites per treatment. Analysis of variance (ANOVA) was applied to assess the effect of the interaction. Descriptive statistics was carried out with SAS ver. 9.0, after checking ANOVA assumptions. Tukey's HSD (Honestly-Significant-Difference) Test was applied with a 95% confidence level.

14.3 Results and Discussion

14.3.1 Nitrogen Content in Leaves

Doses of P and fungi species, as well as their interaction (P dose · fungal species), had significant influence ($p < 0.001$) in N absorption. Foliar N content tended to increase with P doses. In Table 14.2, treatment with 0 kg P_2O_5 ha^{-1} was not included because there was no leaf opposite to the corncob at sampling.

In treatments with P fertilization, the highest level of nitrogen absorption was reached when 54 kg P_2O_5 ha^{-1} was applied (Table 14.2). Several authors have underlined the importance of P, not only in production of fruits and several crops, but also in growth and development of maize (Miller 2000; Harrier and Watson 2003; Smith et al. 2003), therefore, soils with low P contents tend to develop less vigorous plants.

When comparing the mean N absorption due to fungal inoculation, significant differences were observed ($p < 0.001$) among plants with or without AM inoculation. However, this difference in N plant uptake was not observed among mycorrhized treatments. Nevertheless, best absorption was achieved with the native

Table 14.2 Nitrogen content (and %) in the cob opposite leaf of maize inoculated with Glomeromycota fungi grown with different phosphorus doses and harvested in the flowering season

			Treatments				
P_2O_5 (kg ha^{-1})	M0	M1	M2	M3	M4	M5	Mean
27	1.60 b	2.37 a	2.59 a	2.26 a	2.36 a	2.61 a	2.30 A
	(0.33)	(0.39)	(0.08)	(0.16)	(0.24)	(0.22)	
54	1.54 b	2.55 a	2.81 a	2.39 a	2.25 a	2.88 a	2.40 A
	(0.49)	(0.07)	(0.28)	(0.11)	(0.14)	(0.13)	
80	1.55 b	2.39 a	2.25 a	2.59 a	2.49 a	2.57 a	2.31 A
	(0.31)	(0.14)	(0.02)	(0.01)	(0.07)	(0.03)	
Mean	1.56 C	2.43 AB	2.55 AB	2.41 AB	2.36 B	2.68 A	

M0 = No inoculation; M1 = *C. pellucida*; M2 = *S. calospora*; M3 = *A. leptotichum*; M4 = *A. mellea*; M5 = Consortium of native species. Standard deviation in parentheses. Mean comparison test by Tukey ($p < 0.05$). Different lowercase letters indicate significant statistical differences ($p < 0.05$) in the interaction P dose and Glomeromycota inoculum. Different capital letters indicate significant statistical differences ($p < 0.05$) in the interaction P dose (right column) and Glomeromycota inoculum factor (lower row) (coefficient of variation = 9.00%)

species consortium (M5) (Table 14.2). The higher N concentration levels were reached with AM colonized plants, which favor the uptake of N from the soil. Results agrees with previous research (Smith et al. 2003; Barea et al. 2008), who showed that Glomeromycota favor plant nutrients uptake from soil. According to Epstein and Bloom (2005), foliar N content found in this study (1.5–2.8%) indicated no N deficiency, since critical N levels for maize in leaves are below 1.46%. Furthermore, literature reports a diversity of N content in maize leaves. For instance, Reta et al. (2007) found N concentrations of maize stems sowed in soils with low fertility ranging around 1.06–1.14%, far below the concentrations reported in this study. The latter suggests that the effect of the fungi favored and increased the uptake of nutrients from soil, as stated by Smith et al. (2003) and Carrenho et al. (2007) among others.

14.3.2 Phosphorus Absorption

P concentration in the corncob opposite leaf ranged from 0.12% — in the plot with 54 kg P_2O_5 ha^{-1} inoculated with M5 — to 0.16% in the plot with 27 kg P_2O_5 ha^{-1} inoculated with M1. At 27 kg P_20_5 ha^{-1} a better P absorption was achieved (Table 14.3). Data (not shown in this paper) indicated that with a lower dose of phosphorus the AM colonization of maize improved. Although no statistic differences were appreciated in the P dose · fungal species interaction, a slight increase was detected in plots where 27 kg P_2O_5 ha^{-1} were applied. They always showed more absorption of this element, particularly inoculation of M1 which proved to be more efficient. In plots where 54 and 80 kg P_2O_5 ha^{-1} was applied, the mycorrhizae

Table 14.3 Phosphate content (%) in the cob opposite leaf of maize inoculated with Glomeromycota fungi grown with different phosphorus doses, harvested in the flowering season

			Treatments				
P_2O_5 kg.ha^{-1}	M0	M1	M2	M3	M4	M5	Mean
27	0.13 a	0.16 a	0.15 a	0.16 a	0.16 a	0.16 a	0.15 a
	(0.03)	(0.03)	(0.00)	(0.02)	(0.00)	(0.01)	
54	0.13 a	0.13 a	0.13 a	0.13 a	0.13 a	0.12 a	0.13 b
	(0.01)	(0.01)	(0.00)	(0.02)	(0.00)	(0.02)	
80	0.13 a	0.14 a	0.14 a	0.14 a	0.14 a	0.14 a	0.14 ab
	(0.02)	(0.02)	(0.01)	(0.04)	(0.04)	(0.01)	
Mean	0.10 a	0.11 a	0.10 a	0.11 a	0.11 a	0.11 a	0.10

M0 = no inoculation; M1 = C. pellucida; M2 = *S. calospora*; M3 = *A. leptotichum*; M4 = *A.mellea*; M5 = Consortium of native species. Standard deviation in parenthesis. Different lowercase letters indicate significant statistical differences (Tukey, $p < 0.05$) in the interaction P dose and Glomeromycota inoculum (right columm). No significant statistical differences was found among Glomeromycota inocula ($p < 0.05$), according to the comparison test of means by Scheffe (coefficient of variation = 21.12%)

proved to be less efficient in P uptake. It is known that soil must show low P content for the AM functionality, otherwise symbiosis may fail, even if the fungus is able to colonize the root or inoculation is applied (Miller 2000; Harrier and Watson 2003; Sylvia 2013).

In all cases, the values observed in this study are below 0.28 – 0.5% of P as reported by Faggioli and Freytes (2008) in leaf samples of AM-colonized maize, grown in soils with moderate fertility and adequate P contents. As plants absorb P with through the AM symbiotic interaction, while soils show poor supply of this element, very little P can be absorbed without the AM help.

14.3.3 Yield

The analysis of the P dose factor showed significant differences between yield levels ($p < 0.05$), being 54 kg P_2O_5 ha^{-1} the best dose with an average of 3325 kg ha^{-1}. However, no difference was appreciated between the latter and the 80 kg ha^{-1}dose. At 80 kg P_2O_5 ha^{-1} application, 3241 kg ha^{-1} were achieved. However, the dose of 27 kg P_2O_5 ha^{-1} showed the lowest average yield (2100 kgha^{-1}), close that commonly achieved in the zone of Guarico savannas (2000 kg ha^{-1}, Fig. 14.1) (Vielma et al. 2005). This difference shows that applying P increased maize yield and that an intermediate dose, together with AM inoculation, can sustain maize production in these soils (Cardoso and Kuyper 2006; Hernandez et al. 2012).

When considering the fungal factor, statistically significant differences were found ($p < 0.05$). The largest one was observed between inoculated and non-inoculated plants, the latter showing the lowest yield (1458 kg.ha^{-1}). Moreover, M4 (*A. mellea*) showed the best average with a value of 2650 kg ha^{-1}, followed by M5

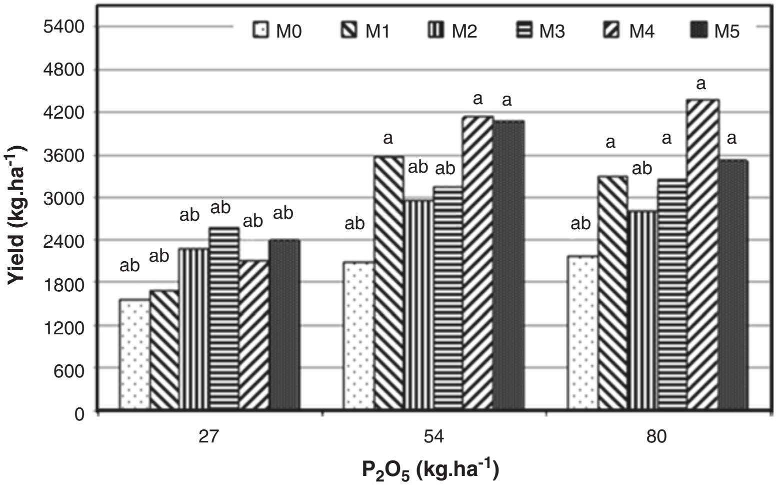

Fig. 14.1 Yield of maize inoculated with arbuscular mycorrhizae and different doses of phosphorus. M0 = no inoculation; M1 = *C. pellucida*; M2 = *S. calospora*; M3 = *A. leptotichum*; M4 = *A. mellea*; M5 = Consortium of native species. Different letters show significant differences ($p < 0.05$), according to Scheffe Test (CV = 27.95%)

(consortium of native species) with 2500 kg ha^{-1}. This suggests that in soils with low phosphoros content, the average production can be enhanced by 30–80%, by applying AM, according to the fungal species and the amount of P in the soil (Carrenho et al. 2007), Sánchez and Velásquez 2008; Barea et al. 2008).

The lowest yields were obtained when no AM inoculation was carried out and/or very low P doses were used (Fig. 14.1). At 27 kg P_2O_5 ha^{-1} with M1 (*C. pellucida)* or M0 treatments, the lowest yields were obtained (1574 and 1691 kg ha^{-1} respectively). These are below the levels reported by Vielma et al. (2005) in acid soils of Venezuela. As the P dose was raised yields improved, even more when AM inoculation was carried out. For instance, with a dose of 54 kg P_2O_5 ha^{-1} and no inoculation, yields of 2076 kg ha^{-1} were reached, but when inoculated with M4 (*A. mellea*) and M5 (consortium of native species), these values increased til 4121 and 4088 kg ha^{-1}, respectively. However, maximum yields were obtained with 80 kg P_2O_5 ha^{-1} and M4 (*A. mellea*, average yield 4384 kg ha^{-1}). These differences are important because when reducing the P application by 33% (27 kg ha^{-1}) in presence of AM, yields drop by 6% only, which means in this case 262.2 kg.

Similar soils showed a yield average of 2670 kg.ha^{-1} (Vielma et al. 2005), an amount surpassed in this study with an intermediate dose of 54 kgP_2O_5 ha^{-1} and AM inoculation. By inoculating with any of the Glomeromycota species essayed, yield averages exceeded. Nevertheless, without inoculation and at the same P doses, this average would be below the value reported by Vielma et al. (2005). Consequently, our results suggest that, in order to obtain an adequate maize yield in these low fertility soils, the use of AM is an option as long as low P doses are used. This agrees with Miller (2000), Harrier and Watson (2003) and Sanchez and Velásquez (2008), which state that P doses in soil can be reduced by using AM. In order to obtain high maize yields, enough amounts of nutrients, particularly P must be present in soil (Epstein and Bloom 2005), as this is one of the most important yield limiting factors, in the studied soils.

Thanks to the efficiency of the Glomeromycota fungi used in this study, the level of 2441 kg ha^{-1} of maize yield obtained by Uribe et al. (2007) was exceeded by inoculations with AM. In this respect, use of arbuscular mycorrhiza represents an opportunity for a sustainable program facing the scarce phosphorus available, a condition typical of soils in the tropics (Cardoso and Kuyper 2006).

14.4 Conclusions

- Using native Glomeromycota fungi, phosphorus fertilization can be reduced by up to 33% without affecting grain production and yields.
- In plantations of AM colonized maize, the raise of P in fertilization had no influence in the capacity of the plant to better absorb this element.
- *Acaulospora mellea* and the consortium of native fungi species have a potential for production of bio-fertilizers based upon Glomeromycota, suitable for maize production in acid soils with low P contents.
- In low fertility soils under savannas weather conditions, maize production can be increased by introducing AM in management plans. *Acaulospora mellea* could be inoculated alone or with other native Glomeromycota, as a consortium.

Acknowledgements The authors express their gratitude to Mrs. Maria Auxi Toro Garcia for manuscript corrections.

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