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Indigenous mycorrhizal fungi from Argentina increase Zn nutrition of maize modulated by Zn fertilization

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Abstract

The effect of nitrogen (N), phosphorous (P), sulfur (S) and zinc (Zn) fertilization on arbuscular mycorrhizal colonization (AMC), nutrition and growth of maize under contrasting tillage management was assessed in a field trial. The effect of Zn fertilization (+Zn: 1.67 mg Zn kg^{-1}, -Zn: 0.33 mg Zn kg^{-1}), and inoculation with four consortium with arbuscular mycorrhizal fungi (AMF) indigenous of two sites of Buenos Aires, Argentina under contrasting managements(Agricultural and Pristine), on arbuscular mycorrhizal colonization (AMC), nutrition and growth of maize was assessed in a greenhouse trial. Zinc fertilization did not affect growth or AMC under field conditions, but in greenhouse, highest dose of Zn depressed AMC. Zinnc application in greenhouse at a reduced dose resulted in symptoms of deficiency which were translated in reduced plant growth but highest mycorrhizal response (MR). The inoculum from Balcarce Agricultural (indigenous from a site with about 19 mg P kg^{-1} and 0.5 mg Zn kg^{-1}) resulted in the highest AMC and MR in both Zn uptake and dry matter production. The inoculum from Coronel Dorrego Pristine (indigenous from a site with about 8 mg P kg^{-1} and 2.2 mg Zn kg^{-1}) was the lowest efficient. We hypothesized that certain soil characteristics could be used to select potentially beneficial inocula to compensate Zn deficiencies in maize.

Keywords: *Zinc, mycorrhizal infectivity, mycorrhizal response, inoculation, native mycorrhizae.*

Introduction

Maize crop in Argentina is, after soybeans, second most important crop, with a planted area of about 2.41 million hectares (FAO, 2011). Non-tillage and contemporary hybrids with high yield generate high nutrients extraction and accumulate high plant residues which cause disturbances in the balance of biological and chemical cycles affecting the P and Zn among others (Ratto and Miguez, 2006). Zinc is required in small but critical concentrations to allow several key plant physiological pathways to function normally. However, Zn deficiency has currently become a global issue since it affects over 50% of agricultural soils (Alloway, 2008). In Buenos Aires province (Argentina) Zn is, after P, N and S, the fourth most important element for the nutrition of maize (Ferraris, 2010) and deficiency in the soil has been detected (Eyherabide *et al*., 2012). Plant uptake of Zn could be altered by edaphic and managements (as P fertilization) factors; also others dependent upon plant-based factors such as production of phytosiderophores, expression of Zn transporters and mycorrhizal associations could affect plant Zn availability (Milani *et al*., 2012; Watts-Williams *et al*., 2014).

While fertilization, in general, negatively affects arbuscular mycorrhizal colonization (AMC), the most extended symbiotic relationship among roots of (up to 80%) vascular plants and soil fungi (Covacevich *et al*., 2007; Smith and Read, 2008), mycorrhizae formation helps in the uptake of low mobility mineral nutrients as P (Smith *et al*., 2011) and Zn (Subramanian *et al*., 2011; Ortas and Akpinar, 2011; Watts-Williams *et al*., 2014). However, evidences of Zn fertilization on AMC, as well mycorrhizal effects on Zn uptake in maize are in most cases contradictory (Cavagnaro, 2008; Subramanian *et al*., 2011; Ortas and Akpinar, 2011). Regarding the importance of Zn deficiency in maize crop and the high diversity of indigenous AMF potentially beneficial (Schalamuk and Cabello, 2010; Thougnon Islas *et al*., 2014) in Argentina, information of this aspect is missing yet but is necessary. In order to clarify this topic, we assessed the effects of Zn fertilization on AMC and the effects of inoculation with indigenous AMF from Argentina on maize Zn uptake and growth.

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Materials and Methods

Field experiment

Field experiment was conducted in 2011-2012 at the Estación Experimental Agropecuaria Instituto Nacional de Tecnología Agropecuaria-Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata (EEA INTA-FCA UNMdP) Station (Balcarce, Argentina 37º 45´S; 58º 18´W) in a field trial which began in 2001. Maize (DK 670) was sown (80,000 plants ha^{-1}) on a complex soil tipic Argiudoll and loamy Petrocalcic Paleudoll. A split plot and complete randomized block design with three replications was assessed with two tillage treatments in the main plot: NT (non-tillage) and CT (conventional tillage). Within sub-plots (5 m x 25 m, row spacing of 0.52 m) following treatments were assessed: unfertilized; NPS= fertilized with N (130 kg N ha^{-1} as urea at V6 (vegetative stage, 6th leaf; according to Ritchie and Hanway, 1982), 40 days after planting –DAP), P (25 kg ha^{-1} as Triple Superphosphate at seedling) and S (11 kg ha^{-1} as $CaSO_4$, 20% S at seedling); NPS Zn= fertilized with N, P, S (as described) and Zn (750 g ha^{-1}, 40% Zn, foliar at V6).

Climatological data were obtained from the EEA-INTA Balcarce (Argentina), located at 3 km from the experiment.

Greenhouse trial

A pot experiment was conducted in greenhouse (EEA INTA Balcarce). Each pot (10 cm diameter and 50 cm in length) was packed with 5 kg of soil-substrate composed by a sterilized (Covacevich and Echeverria, 2003) pristine soil from Balcarce and a substrate (perlite: autoclaved vermiculite, 1:1, V:V) reaching a final soil-substrate ratio of 2:20, V:V. Pots received one pre-germinated (5 days) seedling of maize (DK 670). A factorial design with three replicates was assessed with following treatments: Zn application (+ Zn; - Zn); inoculation (NI= non-inoculated; BA, BP= inocula from Balcarce Agricultural and Pristine, respectively; CDA, CDP = inocula from Coronel Dorrego Agricultural and Pristine, respectively).

Inocula were selected (among 40, characterized in Covacevich *et al*., 2012) for being indigenous of soils with contrasting characteristics in terms of Zn and P availability, organic matter (OM) content and pH (described below). The inocula were previously multiplied (10 weeks in a growth chamber, with ryegrass as plants trap) reaching AMC degree up to 30% and up to 50 spores' g/soil. Inocula consisted spores (belonging to following taxonomic groups: BA= *Funneliformis mosseae*, *Acaulospora denticulata*, *Glomus* sp1, *Scutellospora* sp1, *Gigaspora* sp2; BP= *F. mosseae*, *G. clarum*, *Glomus* sp2, *Scutellospora* sp2; CDA: *F. mosseae*, *A. bireticulata*, *like Scutellospora fulgida*; and CDP: like *F. mosseae*, *Glomus* sp3 and *Acaulospora* sp1, according to INVAM (2012) descriptions, hypha fragment, and piece of mycorrhiza roots were mixed with the soil where they multiplied. Soil pots received 20 g of inocula (placed immediately beneath roots and below the top 5 cm of pots) at sowing and at 7 DAP (10 g in a hole 3 cm close to the maize stem without damaging the roots). Non-inoculated soil pots were mixed with same quantity of sterilized (Covacevich and Echeverria, 2003) inocula and received 20 mL of inocula (Schroeder and Janos, 2004) filtrate (40 g of inocula shaken for 1 h in 400 mL of sterile deionized water filtered in Whatman No. 1).

Pots were periodically rotated and daily irrigated to be maintained at field capacity. Once a week all plants received 20 mL of Hoagland solution (Hoagland and Arnon, 1950) with 50% P. The Zn was added as $ZnSO_47H_2O$ within the Hoagland solution; plants of +Zn treatments: 1.67 mg Zn kg substrate^{-1} (5 mg Zn plant^{-1}); plants of -Zn treatments: 0.33 mg kg Zn substrate^{-1} (1 mg Zn plant^{-1}). Total doses were divided into five weekly applications (each of 0.33 mg Zn kg^{-1} and 0.067 mg Zn kg^{-1} for +Zn and –Zn, respectively).

Field and Greenhouse determinations

Symptoms of Zn deficiency were daily monitored. In the field trial, soil and plant (shoot and root) material were at random (10 plants plot^{-1}) collected at 45 DAP (V6) and also collected at 90 DAP (anthesis or flowering according to Ritchie and Hanway, 1982; 6 plants plot^{-1}). In greenhouse, plant material was collected at 45 DAP and leaf area (LA) was measured (Meter Area Elli-3100, LI-COR, USA). Shoot dry matter production (SDM) and Zn content (Walinga *et al*., 1995) in shoots were measured. In the field, soil and roots were collected (sampler of 5 cm diameter and 20 cm deep, 6 cores/plot= 3 in rows and 3 of the rows). In both experiments, roots were separated from the soil by dry sieving (2 mm) and stained with a trypan blue (0.05%) in distilled water-acid lactic-glycerol (1:1:1) solution according to the modification of Phillips and Hayman (1970) method, in which the phenol reagent was omitted. Arbuscular mycorrhizal colonization (AMC) as well as the frequency of root infection, colonization intensity and arbuscule content were quantified (Brundrett, 2008).

Soil samples were analyzed (Laboratorio de Suelos EEA INTA, Balcarce; for OM content, pH (ratio soil: water 1:2.5) P and Zn availability according to Neville Gough (1996). Most Probable Number (MPN) of aerobic

bacterial Colony Forming Units (CFU) was assessed according to FAO (1967) at the end of pot experiment.

For each inoculation treatment and Zn fertilization (greenhouse trial) mycorrhizal responsiveness (MR) was separately calculated according to Cavagnaro *et al*. (2003) by using the individual SDM production of inoculated (I) plants and mean SDM of non inoculated (NI) plants (Equation 1).

MR = [SDM(I) – mean SDM (NI)/ Mean SDM (NI)] x 100

The MR was similarly calculated for Zn uptake and leaf area.

Data analysis

Tested variables (in soil: pH, Zn, P, OM, SDM and CFU; in plant: Zn content and uptake, mycorrhizal colonization intensity and arbuscule content) were analyzed by analysis of variance (ANOVA), means were separated by the Least Significance Difference test (LSD p <0.05) by using the SAS software (2009). Irrespective of the significance of the interactions, in the field trial, we examined the effect of fertilization treatments separately for each management and sampling. In the greenhouse trial, we showed the effect of inoculation separately for each Zn supply condition.

Results and Discussion

Climatic and soil conditions

The soil water content in the field was below 50% of available water from V6 (vegetative period) to flowering, approaching the wilting point; and temperature and relative humidity remained close to historical (data not shown). Tillage did not affect pH, Zn, P and OM contents and P fertilization increased the soil P content but did not affect the pH, Zn and OM (Table 1).

The four inocula tested in greenhouse had contrasting chemical and microbiological characteristics (Table 2). Tested inocula had high AMC and spore density than others tested by Thougnon Islas *et al*. (2014) which were also indigenous from the Buenos Aires Province (others locations), Argentina, and similarly multiplied. The soil-substrate (before starting experiment) had 1.72 mg Zn kg^{-1}. The pH and the OM content of soil pots showed no changes after Zn fertilization or inoculation; and P content at the end of the trial was generally higher than the content before planting (Table 3), mainly in the NI treatments as well as in the –Zn/CDP ones which also had highest soil Zn content. The total CFU of bacteria of substrate pots at the end of the trial did not change after Zn fertilization (4.28 10^5 and 3.95 10^5 for -Zn and +Zn, respectively). Pots inoculated with BA showed higher bacteria CFUs (Table 2), which nearly doubled in other treatments; and inoculated with CDP showed lowest bacteria CFUs. The NI pots showed intermediate bacteria CFUs as well as BP and CDA ones. Microbial communities in the inocula belonged mainly to Gram +ve and Gram -ve bacteria, yeast and Actinomycetes and no obvious differences among inocula were detected (data not shown).

Mycorrhizal colonization

The AMC (mycorrhizal intensity and arbuscules content) was high at the vegetative maize stage reaching 60% and then it decreased at flowering (Figure 1). In Argentina, Faggioli and Freytes (2008) found lower AMC of maize in V6 (reaching 50%) which, contrarily with ours results, increased until flowering. It is probably that water stress recorded in flowering at our field experiment contributed to the depression of general AMC at this period. In V6, fertilization or tillage did not significantly affect native AMC, and it was in concordance with reports of Faggioli and Freytes (2008). Irrespective of fertilization or tillage, highest AMC of maize found under field conditions could be an indicator of high indigenous mycorrhizal activity at the studied Argentinean agricultural soil. The NPS fertilization depressed AMC to a greater extent than NPSZn fertilization mainly under CT at flowering (Figure 1). Depressive effects of P fertilization on AMC are well reported (Siddiqui *et al*., 2008; Smith and Read, 2008). Reductions in colonization with increasing soil P may be due to a reduction in fungal growth as well as to increases in the growth of roots (Cavagnaro, 2008; Smith and Read, 2008). Some reports (Gildon and Tinker, 1983; Boyle and Paul, 1988) showed depressive effect of Zn on AMC under field conditions. Cavagnaro (2008) suggested a diverse range of responses of AMC to Zn addition and stated that presumably similar mechanisms operate with respect to addition of P and reduced colonisation can at least in part be due to reduced growth of the fungus, in addition to increased root growth. Bi *et al*. (2003) stated that the addition of P to the soil can modify effects of Zn on AMC, and showed reductions in the AMC in low (no added P) P (from 35, 38 to 14%) but not high (100 mg kg^{-1} added P) P (from 22, 15 to 20%) treatments following addition of Zn over a wide concentration range (0, 50 and 400 mg Zn kg $soil^{-1}$). At our experiment, plants receiving Zn and P appeared to increase slightly (but not significantly) in AMC in relation to P alone. The lack of depressive effects of Zn fertilization on AMC may be due to short elapsed period of time between Zn fertilization and sampling of plant material (maize was Zn fertilized at 40 DAP and sampling V6 was performed at 45 DAP) and also because the Zn fertilization history (since 2001) did not affect the Zn content in the soil (Table 1).

Table 1: Chemical soil analysis at seeding and during the maize crop growth in the field experiment. Shoot dry matter (SDM), concentration and uptake of zinc (Zn conc and Zn upt, respectively) of maize crop in field

Period	Tillage	Fertilization	Soil				Shoot		
			pH	Zn	P	OM	SDM	Zn conc	Zn upt
				(mg kg soil^{-1})		(%)	(kg ha^{-1})	mg kg^{-1})	(g ha^{-1})
Seeding	CT	Unfertilized	6.1 a	2.0 a	13.9 b	4.6 a			
		NPS	6.0 a	1.9 a	34.1 a	5.1 a			
		NPS Zn	5.9 a	2.0 a	38.0 a	4.7 a			
	NT	Unfertilized	6.1 a	2.1 a	13.7 b	4.6 a			
		NPS	6.0 a	2.0 a	31.9 a	4.9 a			
		NPS Zn	6.0 a	2.2 a	28.6 a	5.0 a			
V6	CT	Unfertilized	6.0 a	nd	16.4 b	4.6 a	510 a	56.2 a	28.9 b
		NPS	5.8 a	nd	37.7 ab	5.9 a	580 a	59.8 a	33.6 ab
		NPS Zn	5.7 a	nd	48.7 a	4.7 a	630 a	74.1 a	44.5 a
	NT	Unfertilized	6.3 a	nd	15.8 b	4.6 a	306 a	35.5 a	9.3 a
		NPS	5.6 a	nd	52.5 a	5.2 a	261 a	43.2 a	13.5 a
		NPS Zn	5.6 a	nd	33.8 ab	4.8 a	264 a	62.0 a	16.4 a
Anthesis	CT	Unfertilized	6.2 a	nd	18.6 b	4.8 a	12344 b	nd	nd
		NPS	6.1 a	nd	31.2 ab	6.2 a	16789 a	nd	nd
		NPS Zn	5.9 a	nd	41.7 a	5.1 a	14732 a	nd	nd
	NT	Unfertilized	5.9 a	nd	24.9 b	5.4 a	13594 a	nd	nd
		NPS	5.0 a	nd	36.6 a	5.3 a	12838 a	nd	nd
		NPS Zn	5.9 a	nd	31.3 ab	5.6 a	11196 a	nd	nd

(CT: conventional tillage; NT: non- tillage; OM: organic matter; nd: not determined); For each period and tillage, means with different letter (s) in columns are significantly different between fertilization treatments at p <0.05

Table 2: Chemical (pH, Zn, Bray-P, OM) soil characteristics of chemical characteristics of the soil from which the inocula were collected. Mycotrophic characteristics (F = frequency of mycorrhization, M = mycorrhizal colonization intensity, A = arbuscule content and spores density) of tested inocula. Bacterial counting in maize plants growth substrates in the greenhouse trial

	Before inoculation								After inoculation
Inoculation	pH	Zn (mg kg soil^{-1})	P	OM	F	M	A	Spores density (N°spores AMF g soil^{-1})	Total Bacterial (CFU g soil^{-1})
					(%)				
NI									4.305 10^5 b
BA	6.1	0.5	18.3	5.1	58	22	8	54	6.273 10^5 a
BP	6.0	14.0	88.0	8.4	52	10	2	52	3.395 10^5 b
CDA	6.7	0.3	25.9	2.8	52	18	7	59	3.312 10^5 b
CDP	8.1	2.2	7.7	3.2	30	9	3	57	1.623 10^5 c

Inoculation treatments NI= non inoculated; B= Balcarce; CD= Coronel Dorrego; A= Agricultural; P= Pristine; CFU: Colony Forming Units. Means with different letter (s) in columns are significantly different between inoculation treatments at P <0.05

Inoculation at the greenhouse trial resulted in all cases in detectable AMC of maize roots (Figure 2) and there were no structures of AMF in the NI maize roots. Lowest AMC was found in roots of +Zn plants (Fig. 2, insert). At each level of Zn, highest AMC was found after BA inoculation and +Zn depressed AMC in relation to the –Zn treatment. Also, Liu *et al*. (2000) showed depressive effect of 0.24 and 0.48 mg Zn kg^{-1} plus Fe, Mn, Cu, Mo on AMC of maize. Contrarily, Watts-Williams *et al*. (2014) found no depressive Zn applications effect on AMC of tomato, however it was dependent on cultivars. We found depressive effect of the application of Zn even at doses lower than those reported by Goh *et al*. (1997) who found that

AMC of inoculated wheat plants decreased significantly by 2.5 and 10 mg Zn kg^{-1} alone and in combination with P. Our results did not agree with McIlveen and Cole (1975) who found that AMC of soybean plants was enhanced by 18 mg Zn kg^{-1} but rates of 45 and 135 mg Zn kg^{-1} resulted in decreased infection. Contradictory findings have also been reported by Subramanian *et al.* (2009) who found no evidence of mycorrhizae depression of maize roots by addition of 0-5.0 mg Zn kg^{-1}. Besides discrepancies found in previous reports no uniformity between field and greenhouse could be partly due to the fact in the field the Zn application was foliar and did not affect the soil Zn content while in the greenhouse the application was made to the substrate and Zn differences were detected in the Zn content in the substrate.

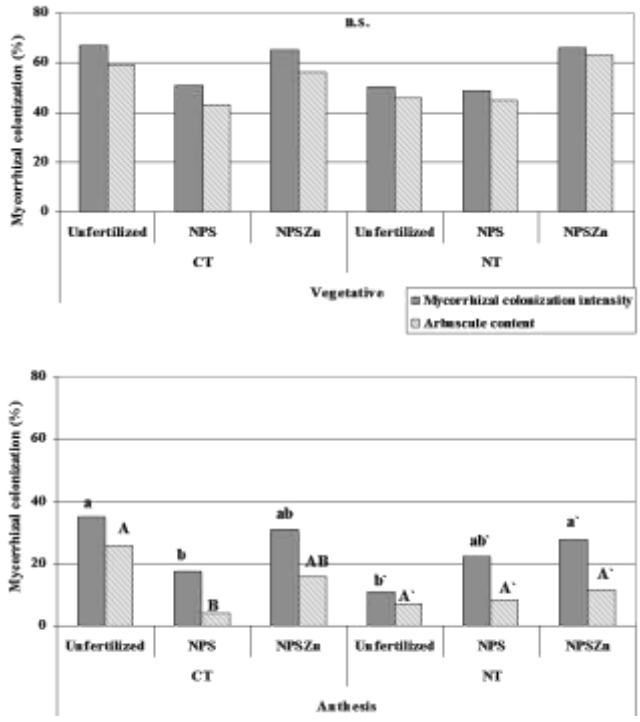

Figure 1: Mycorrhizal colonization (mycorrhizal colonization intensity and arbuscule content) of maize roots during vegetative (V6) and anthesis period in the field trial

CT: Conventional tillage, NT: non-tillage; ns: no significant differences among treatments; For each tillage, means with different letter (s) in bars are significantly different among fertilization treatments at p <0.05

Growth, Zn uptake and mycorrhizal response of maize plants

In field, there were no visual symptoms of Zn deficiency but in greenhouse the NI/–Zn plants showed whitish longitudinal bands in the young leaves (Figure 3) during the first two weeks of growth. Soils having Zn contents less than 0.7-0.8 mg kg^{-1} are generally responsive to Zn fertilizer for maize crop (Kuldeep, 2009). Jones (1998) determined that below 20 mg Zn kg^{-1} in plant, deficiency symptoms appear in maize young leaves. At our study the Zn content of soil pots at the beginning of the trial was about 1.72 mg kg^{-1} and plant Zn content at 45 DAP was about 21-28 mg kg^{-1}. Both soil and plant Zn content were above reported thresholds, even so, Zn deficiency symptoms (interveinal chlorosis, as described by Ratto and Miguez, (2006) were found.

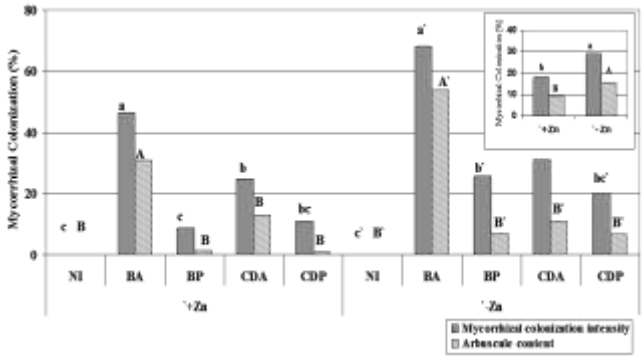

Figure 2: Mycorrhizal colonization (mycorrhizal colonization intensity and arbuscule content) of maize roots in the greenhouse trial.

Larger figure: For each fertilization treatment, means with different letter (s) in bars are significantly different among inoculation treatments at p <0.05. Insert Figure: means with different letter (s) in bars are significantly different between fertilization treatments at p <0.05. Descriptions of treatments are in Tables 2 and 3. NI: uninoculated.

Although SDM, Zn uptake and LA were not significantly affected (p <0.05) by Zn fertilization (Table 1), some increases in Zn uptake were obtained for plants growing under CT and received Zn. Similarly, Barbieri *et al.* (2012) found no significant effect of Zn application of maize crop under NT in Balcarce. Although Zn applications were made during more than 10 years at this field trial, soil Zn content was not affected by Zn application and remained in all cases over the threshold of deficiency (Table 1) which explains the lack of response to Zn application.

Inoculation was performed only in pots at the greenhouse trial. For each Zn fertilization condition, inoculation did not significantly increase SDM or LA (Table 3). Highest Zn concentration and uptake were found in +Zn/BA plants and contents agree with those found by Barbieri *et al.* (2012) for maize growing in field. Highest MR in SDM, Zn uptake and LA were recorded in –Zn/BA plants. Although Ortas (2012) also found possitive effects

Table 3: Chemical soil and substrate characteristic at the greenhouse trial. Shoot dry matter (SDM), leaf area (LA), concentration and uptake of zinc (Zn conc and Zn upt, respectively) and mycorrhizal response (MR) of maize plants at 45 DAP

Condition			pH		P	OM	SDM	MR (SDM)	LA	MR (LA)	Zn conc	Zn upt	MR (Zn upt)
					(mg kg soil^{-1})	(%)	(g plant^{-1})	(%)	(cm^2 plant^{-1})	(%)	(mg kg^{-1})	(mg plant^{-1})	(%)
Fertilization	Inoculation	Zn											
Soil BF	--	--	5.9	6.6	27.3	4.7							
Substrate BS	--	--	6.6	1.7	17.9	1.7							
Substrate AS	+Zn	NI	8.6 a	3.3 b	28.1 a	3.2 a	5.5 a	--	944 a	--	28 b	0.15 b	--
	+Zn	BA	8.2 a	3.2 b	15.3 b	2.5 a	6.0 a	9.8	1091 a	15.5	36 a	0.22 a	46.3
	+Zn	BP	7.9 a	3.4 b	21.7 ab	2.8 a	5.4 a	-1	958 a	1.5	27 b	0.15 b	-0.9
	+Zn	CDA	8.6 a	3.0 b	20.1 ab	2.2 a	4.7 a	-14.8	917 a	-2.9	25 b	0.12 b	-22.2
	+Zn	CDP	8.3 a	6.3 a	17.7 b	2.4 a	4.9 a	-11.3	933 a	-1.2	24 b	0.12 b	-23.3
	-Zn	NI	8.5 a	3.1 a	26.1 ab	2.3 a	4.1 a	--	856 a	--	28 a	0.11 a	--
	-Zn	BA	8.5 a	2.6 a	22.9 b	2.7 a	5.8 a	41.4	1049 a	22.6	25 ab	0.14 a	25.1
	-Zn	BP	8.5 a	2.6 a	17.8 b	2.7 a	4.9 a	21.1	877a	2.4	27 a	0.13 a	16.5
	-Zn	CDA	8.2 a	2.8 a	19.6 b	2.6 a	5.5 a	34.1	950 a	10.9	23 ab	0.13 a	10.6
	-Zn	CDP	8.0 a	2.7 a	36.9 a	2.8 a	5.3 a	30.1	835 a	-2.5	21 b	0.11 a	-3.1

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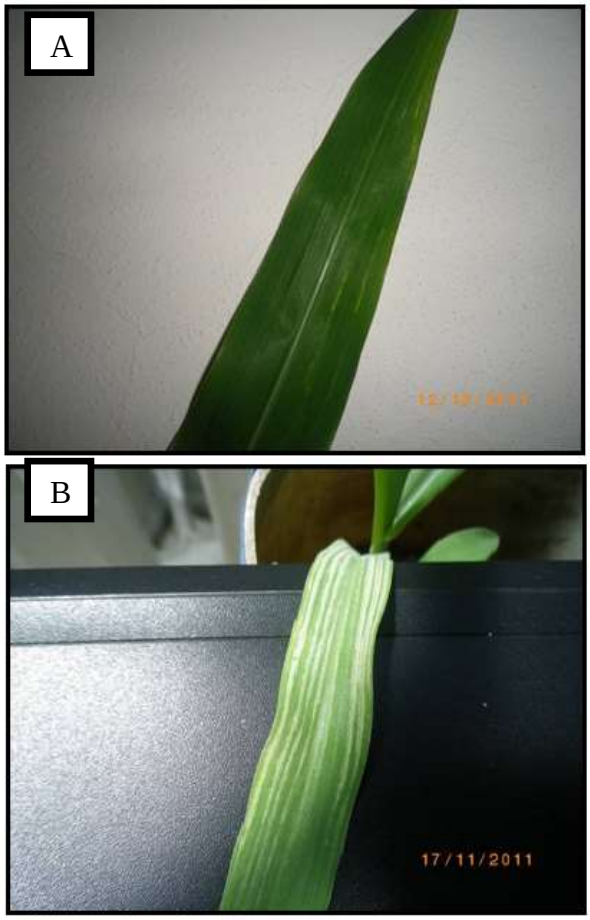

Photo 1: Zn deficiency in maize for the NI-Zn treatment (A) and maize without symptoms for the NI+Zn treatment (B)

determined values of Zn plant concentration were higher than reported by Ortas (2012). In general, +Zn plants showed negative MR for tested plant parameter. Schroeder and Janos (2004) found growth depressions in the first month of growth of maize plants inoculated with AMF native of Costa Rica but by the second month depressing effects disappeared. Subramanian *et al*. (2009) have also found MR decreases in SDM production of maize plants with increases with low Zn. With our results we would may assume that in these cases the plant-AMF relationship has become from mutualistic symbiosis (under-Zn) to parasitic (under +Zn). Positives MR under –Zn conditions could be associated to that reported by Sharma *et al*. (1992) who found that kinetics of Zn absorption by mycorrhizal maize was greater (sigmoidal) at low concentrations (less than 4 mmol m^{-3}) but decreased (obeying the Michaelis-Menten kinetics) at higher (4.0 mmol to 1.07 mol m^{-3}) levels. The arbuscular hypha is probably the main site for the nutrient exchange between fungi and plant, and arbuscule content has been reported as a parameter of AMF efficiency (Covacevich and Echeverría, 2010). In our study, most efficient inocula (BA) showed highest arbuscule content even in the condition of Zn fertilization in which all colonization was depressed.

Growth increases in maize by inoculation with AMF under controlled conditions have been well reported (Liu *et al*., 2000; Schroeder and Janos, 2004; Subramanian *et al*., 2009). Sharif and Jan (2008) found growth and Zn uptake increases after inoculation with AMF (mainly belonging to *Glomus fasciculatum* and in lower extent to other five AMF species). At our study, the most efficient inocula (BA) showed a probable higher diversity than others, but it still requires further confirmation and re-identification. Ortas and Akpinar (2011) reported that mycorrhizal dependency (MD, Plenchette *et al*., 1983) in six maize genotypes was in the range of -4 to 51% after inoculation with native AMF from Turkey (Balcal series) and 4 to 39% by inoculation with a cocktail composed of seven AMF. Our results would match with MD values in the range of 15 to 29% for –Zn, and of -26 to 6% for +Zn plants. Ortas (2012) also stated that maize and green pepper were highly mycorrizal dependent (MD) after inoculation with *F. moseae and G. etunicatum*, mainly under both low P and Zn supply and that mycorrhizae play an essential role in P and Zn nutrition of plants in P and Zn-deficient soils. Contrarily, Karasawa *et al.* (2001) did not found growth increases of maize plants inoculated with several species of *Glomus* and associated the lack of MR to deleterious effect on AMF produced by some chemical compounds after soil autoclaving prior to inoculation. Erradication of AMF by formaldehyde (Covacevich and Echeverria, 2003) at our study did not adversely affect AMF multiplication or growth of inoculated plants. Sharif and Jan (2008) reported that although inoculation of maize with indigenous AMF of Pakistan (mainly *G. fasciculatum= Rhizophagus fasciculatus*) increased shoot and root dry matter, did not significantly increase the Zn uptake of maize plants. Contrarily, Subramanian *et al.* (2009) reported positive MR in Zn uptake of maize plants at 45 DAP under greenhouse conditions and MD between 19-16% for Zn uptake after inoculation and supplies of Zn in the range of 0 to 2.5 mg kg^{-1} soil. We obtained positives MR for Zn uptake and our results correspond to a MD for Zn uptake in the range of -14 to 27%, suggesting a promising contribution of indigenous mycorrhizae from Argentina for maize Zn nutrition.

Conclusions

Foliar Zn application in field did not increase soil nor plant Zn content and growth, and did not affect AMC of maize, probably associated to a soil Zn content above

deficiency thresholds. However, soil Zn fertilization negatively affected AMC and mycorhizal responsiveness in greenhouse. We speculate that the soil Zn content could act as modulator of mycorrhizae formation and effectiveness of maize under soil Zn deficiency. Results of this study provide the first evidence that a microbial consortium with native AMF from agricultural soils of Balcarce (with higher P but lower Zn content) developed greatest AMC and was the most effective inoculum for Zn uptake and growth of maize. Lowest effective inoculum was indigenous from a pristine site of Coronel Dorrego (with lower P but higher Zn content). More detailed studies of the diversity of inocula used are necessary and future studies should focus to test its effectiveness in substrates with native microbial populations (non-sterile substrates) as well as against different P fertilizer supplies.

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References

Alloway, B.J. 2008. Zinc in Soils and Crop Nutrition. 2nd Ed. IZA and IFA, Brussels, Belgium and Paris, France.

Barbieri, P.A., H.E. Echeverría, H.R. Sainz Rozas, E. Miqueo and S.E. Silva. 2012. Corn crop response to zinc application in Balcarce. *In:* XIX CL - XXIII CACdS, Argentina. Available online at: http://www.congresodesuelos.org.ar/site/posters/Resumenes/0801.pdf [Accessed on: 13/06/2013].

Bi, Y.L., X.L. Li and P. Christie. 2003. Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus. *Chemosphere* 50: 831–837.

Boyle, M. and E.A. Paul. 1988. Vesicular arbuscular mycorrhizal associations with barley on sewage amended plots. *Soil Biology and Biochemistry* 26: 1117–1124.

Brundrett, M. 2008. Mycorrhizal associations: The web resource. Section 10. Methods for identifying mycorrhizas. Available online at http://mycorrhizas.info/method.html [Accessed on: 08.06.2011].

Cavagnaro, T.R. 2008. The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: A review. *Plant and Soil* 304: 315–325

Cavagnaro, T.R., F.A. Smith, S.M. Ayling and S.E. Smith. 2003. Growth and P nutrition of a Paris-type arbuscular mycorrhizal symbiosis. *New Phytologist* 157: 127–134.

Covacevich, F. and H.E. Echeverria. 2003. Use of formaldehyde for the eradication of arbuscular mycorrhizal fungi in soil samples. *Ciencia del Suelo* 21: 9–17.

Covacevich, F., H.E. Echeverría and L.A.N. Aguirrezabal. 2007. Soil available phosphorus status determines indigenous mycorrhizal colonization into field and glasshouse-grown spring wheat in Argentina. *Applied Soil Ecology* 35: 1–9.

Covacevich, F. and H.E. Echeverría. 2010. Indicadores para seleccionar inóculos de hongos micorricicos arbusculares eficientes en suelos moderadamente ácidos. *Ciencia del Suelo* 28: 9–22.

Covacevich, F., M. Eyherabide, H.R. Sainz Rozas and H.E. Echeverría. 2012. Capacidad micotrófica arbuscular y características químicas de suelos agrícolas y prístinos de Buenos Aires (Argentina). *Ciencia del Suelo* 30: 119–128.

Eyherabide, M., H.R. Sainz Rozas, H.E. Echeverría, J. Velasco, M. Barraco, G. Ferraris and H. Angelini. 2012. Zinc levels in soils of the argentine Pampas. *In:* XIX CL - XXIII CACdS, Argentina. Available online at: http://www.congresodesuelos.org.ar/site/posters/Resumenes/0798.pdf [Accessed on: 13/06/2013].

Faggioli V.S. and G.S.Freites 2008. Micorrización natural de maíz y su relación con la absorción de fósforo del suelo en diferentes sistemas de labranzas y fertilización XXI Congreso Argentino de la Ciencia del Suelo. Available online at: http://inta.gob.ar/documentos/micorrizacion-natural-de-maiz-y-su-relacion-con-la-absorcion-de-fosforo-del-suelo-en-diferentes-sistemas-de-labranzas-y-fertilizacion/at_multi_download/file/INTA-Micorrizaci%C3%B3n%20natural%20de%20ma%C%ADz%20.pdf [Accessed on: 07/04/2013].

FAO. 1967. A practical manual of soil microbiology laboratory methods. p. 68. Available on line at http://www.fao.org/docrep/018/aq359e/aq359e.pdf [Accessed on: 02/07/2013]

FAO. 2011. United Nations Organization for Food and Agriculture. Available online at: http://www.fao.org/agronoticias [Accessed on: 05/06/2014].

Ferraris, G. 2010. Micronutrientes en cultivos extensivos. Necesidad actual o tecnología para el futuro? Available on line : http://www.profertilnutrientes.com.ar/images/archivos/559.pdf [Accessed on: 18/01/2013].

Gildon, A. and P.B. Tinker. 1983. Interactions of vesicular arbuscular mycorrhizal infections and heavy metals in Dodd plants. II. The effects of infection on uptake of copper. *New Phytologist* 95: 263–268.

Goh, T.B., M.R. Banerjee, S. Tu and D.L. Burton.1997. Vesicular arbuscular mycorrhizae-mediated uptake and translocation of P and Zn by wheat in a calcareous soil. *Canadian Journal of Plant Science* 77: 301–305.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* 347:1–32.

INVAM. 2012. International culture collection of (Vesicular) *Arbuscular Mycorrhizal* Fungi. Available on line at http://www.invam.wvu.edu/the-fungiclassification/glomaceae [Accessed on: 08/06/2011].

Jones, J.B. 1998. Plant Nutrition Manual. CRC Press LLC. 149p.

Karasawa, T., Y. Kasahara and M. Takebe. 2001. Variable response of growth and arbuscular mycorrhizal colonization of maize plants to preceding crops in various types of soils. *Biology and Fertility of Soils* 33: 286–293.

Kuldeep, S. 2009. The critical zinc deficiency levels in Indian soils and cereal crops. *In:* The Proceedings of the International Plant Nutrition Colloquium XVI. UC Davis. Available online at: http://www.escholarship.org/uc/item/4h0788h5#page-2 [Accessed on: 20/08/2011].

Lindsay, W.L. and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of American Journal* 42: 421–428.

Liu, A., C. Hamel, R.I. Hamilton, B.L. Ma and D.L. Smith. 2000. Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* 9: 331–336.

McIlveen, W.D. and H. Cole Jr. 1979. Influence of zinc on development of the endomycorrhizal fungus *Glomus mosseae* and its mediation of P uptake by *Glycine max* 'Amsoy 71'. *Agriculture and Environment* 4: 245–256.

Milani, N., M.J. McLaughlin, S.P. Stacey, J.K. Kirby, G.M. Hettiarachchi, D.G. Beak and G Cornelis. 2012. Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles. *Journal of Agricultural and Food Chemistry* 60: 3991–3998.

Neville Gough, P.Ag. 1996. Soil and plant tissue testing methods and interpretations of their results for de British Columbia agricultural soils. 85 p. Available on line at: http://www.agf.gov.bc.ca/resmgmt/publist/600Series/634200-3_Soil_and_Plant_Tissue_Testing_Methods.pdf [Accessed on: 05.06.2014].

Ortas, I. 2012. Do maize and pepper plants depend on mycorrhizae in terms of phosphorus and zinc uptake? *Journal of Plant Nutrition* 35:1639–1656.

Ortas, I. and C. Akpinar. 2011. Response of maize genotypes to several mycorrhizal inoculums in terms of plant growth, nutrient uptake and spore production. *Journal of Plant Nutrition* 34: 970–987.

Phillips, J.M. and D.S. Hayman. 1970. Improves procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55: 158–161.

Plenchette, C.A., A. Fortin and N. Forlan. 1983. Growth response of several plant species to mycorrhiza in a soil of moderate P-fertility. I. Mycorrhizae under field conditions. *Plant and Soil* 70: 199–203.

Ratto, S.E. and F.H. Miguez. 2006. Zinc en el cultivo de maíz, deficiencia de oportunidad. *Informaciones agronómicas* 31: 11–14.

Ritchie, S.W. and J.J. Hanway. 1982. How a corn plant develops. Iowa State University of Science and Technology. Special Report 48. Cooperative Extension Services, Am, Iowa. 21p.

Sharif, M. and B. Jan. 2008. Growth and nutrients accumulation of maize plants as affected by the inoculation of arbuscular mycorrhizal fungi with rock phosphate. *Soil and Environment* 27: 116–122.

SAS Institute Inc. 2009. SAS/STAT® 9.2. User's Guide. 2nd Ed. SAS Institute Inc., Cary.

Schalamuk, S. and M. Cabello. 2010. Arbuscular mycorrhizal fungal propagules from tillage and no-tillage systems: possible effects on Glomeromycota diversity. *Mycologia* 102: 261–268.

Schroeder, M.S. and D.P. Janos. 2004. Phosphorus and intraspecific density alter plant responses to arbuscular mycorrhizas. *Plant and Soil* 264: 335–348.

Sharif, M. and B. Jan. 2008. Growth and nutrients accumularion of maize plants as affected by the inoculation of Arbuscular Mycorrhizal fungi with rock phosphate. *Soil and Environmnent* 27: 116–122.

Sharma, A.K., P.C. Srivastava, B.N. Johri and V.S. Rathore. 1992. Kinetics of zinc uptake by mycorrhizal (VAM) and non-mycorrhizal maize (*Zea mays* L.) roots. *Biology and Fertility of Soils* 13: 206–210.

Siddiqui, Z.A., M.S. Akhtar and K. Futai. 2008. Mycorrhizae: Sustainable Agriculture and Forestry, Springer, The Netherlands. 362p.

Smith, S.E. and D.J. Read. 2008. Mycorrhizal Symbiosis. Elsevier, New York. 800p.

Smith, S.E., I. Jakobsen, M. Grønlund and F.A. Smith. 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.

Subramanian, K.S., V. Tenshia, K. Jayalakshmi and V. Ramachandran. 2009. Role of arbuscular mycorrhizal fungus (*Glomus intraradices*) – (fungus aided) in zinc nutrition of maize. *Journal of Agricultural Biotechnology and Sustainable Development* 1: 029–038.

Subramanian, K.S., V. Tenshia, K. Jayalakshmi and V. Ramachandran. 2011. Antioxidant enzyme activities in arbuscular mycorrhizal (*Glomus intraradices*) fungus inoculated and non-inoculated maize plants under Zn deficiency. *Indian Journal of Microbiology* 51: 37–43.

Thougnon Islas A.J., M. Eyherabide, H.E. Echeverría, H.R. Sainz Rozas and F. Covacevich. 2014. Capacidad micotrófica y eficiencia de consorcios con hongos micorrícicos nativos de suelos de la Provincia de Buenos Aires con manejo contrastante. Revista Argentina de Microbiologia. (In Press).

Walinga, I., J.J. Van Der Lee, V.J.G. Houba, W. Van Vark and I. Novozamsky. 1995. Plant Analysis Manual. Kluwer Academic Publishers.

Watts-Williams, S.J., T.W. Turney, A.F. Patti and T.R. Cavagnaro. 2014. Uptake of zinc and phosphorus by plants is affected by zinc fertilizer material and arbuscular mycorrhizas. *Plant and Soil* 376: 165–175.