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Receptivity of an Argentinean Pampas Soil to Arbuscular Mycorrhizal *Glomus* and *Acaulospora* Strains

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Abstract: Soil receptivity to arbuscular mycorrhizal (AM) fungi tests the capacity of a soil to favour the mycorrhizal development after inoculation. Thus, receptivity is a key criterion to assess whether the introduction of non-indigenous AM fungi will successfully improve plant growth. Two experiments were set up to investigate the receptivity of a moderately acidic wheat-growing soil of the Argentinean Pampas (south America) to non indigenous *Glomus* and *Acaulospora* AM strains. Soil was collected from agricultural non fertilized wheat fields and native AM fungi were identified. At first, soil was tindalized and four AM strains were study in their capacity of colonizing and improving growth of a highly mycotrophic and mycorrhizal responsive test plant. Then, the most efficient AM strains were inoculated in the soil containing the native microflora and fertilized with phosphorus (P) and the mycorrhizal development and benefit were assessed for wheat plants. The *G. clarum* fungus formed the highest colonization, as revealed by trypan blue (TB) staining and for alkaline phosphatase (ALP) activity in onion plants, when it was inoculated in the sterile soil and intermediate in the presence of indigenous AM fungi, but it did not produced highest mycorrhizal responsiveness (MR) of wheat. The lowest colonization and non significant increases in plants growth were found after inoculation with *A. laevis*. Thus, neither strain was efficient at developing in the soil and improving plant growth. Inoculation with *G. claroideum* or *A. longula* led to intermediate colonization of onion when the fungus was inoculated in the sterile soil and in wheat in the native fertilized soil. Although fertilization depressed AM development, colonization and arbuscules were highest for plants inoculated with *G. claroideum* and *A. longula*. With nil P, colonization was highest for plants inoculated with *G. claroideum* followed by *G. clarum* and non inoculated (native mycorrhizal) plants. Shoot dry matter of wheat was really increased upon P fertilization, but it was only sporadically increased by inoculation – for shoots and roots only for *G. claroideum* at low P and for shoots for *A. longula* at high P-. Mycorrhizal responsiveness was higher with nil P in comparison with added P. Inoculation with *G. claroideum* and *A. longula* plus fertilization caused the greatest MR in shoot and grain dry matter and in P uptake compared to non-inoculated plants. This is the first report on the soil receptivity to non indigenous AM fungi in the Argentinean soil. Further research must confirm if the inoculation of field agricultural soils from Argentinean Pampas without indigenous *A. longula* or *G. claroideum* strains and moderate P fertilization, could enhance the development of an effective AM symbiosis for wheat crops.

Key words: Soil receptivity · Arbuscular mycorrhiza · Mycorrhizal responsiveness · *Glomus* sp. · *Acaulospora* sp. · Wheat · Onion

INTRODUCTION

The growth enhancement of plants colonized by arbuscular mycorrhizal (AM) fungi is a well-known process [1, 2]. Although nearly all soils contain indigenous AM fungi, the association between a given plant species and the indigenous AM population may not always be optimal [3, 4]. To increase or maintain productivity without damaging the soil and the environment, an efficient use of fertilizer is required. Thus, inoculation with an effective AM fungus could be a way of enhancing plant growth and at the same time could reduce the costs associated with fertilizer application and risks of environmental pollution.

Wheat is a global food supply and is one of the most important crops in Argentina, with an

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average global production of 14.9 million tonnes over the last 5 yr. Thirty percent of this amount is produced in the southeastern part of the humid Argentinean Pampas. Soils of the southern Buenos Aires province are moderately acid, usually contain 60-80 g kg^{-1} organic matter content and have low concentrations (6-8 mg kg^{-1}) of native available phosphorus (P) [5]. Thus, fertilization with P (15-25 kg P ha^{-1}) and nitrogen (N, 120 kg N ha^{-1}) are common practices for farmers in order to increase wheat yield. Additionally, the soils of this region contain indigenous AM fungi that colonize both wild and cultivated plants [6, 7, 8]. In previous work [9] it was found that the presence of indigenous AM fungi in such soil did not improve wheat growth (25% lower biomass in indigenous mycorrhizal wheat plants than in benomyl mycorrhizal suppressed counterparts). However, the AM-based inoculants are not included in the farming systems of Argentina and fertilizers applications are the main cost of the wheat crops.

Improving wheat yield and maintaining soil sustainability are issues of particular interest for wheat growers having agricultural fields with non-efficient AM indigenous population. Although wheat is not highly dependent on mycorrhiza, the growth response to inoculation depends on wheat cultivar [10, 11]. Some positive influence of AM colonization on wheat yield has been reported following inoculation [12, 13] particularly in low-P soils [14, 15]. We hypothesised that wheat soil inoculation with non-indigenous AM fungal strains could be an effective alternative to P fertilization for improving wheat growth and yield.

Soils differ in their receptivity to micro-organisms when non-native strains are introduced in an ecosystem. In particular concerning the AM fungi receptiveness (i.e. the capacity of the soil to favour AM fungal development after inoculation), appears to be one of the most fundamental properties of soils [4]. Thus, the development of a test for evaluating soil receptivity would be a key for reliable assessment of successful introduction of non-indigenous AM fungi into a soil. Plenchette [4] estimated soil receptiveness in a gamma irradiated soil for a unique AM fungal strain by a dose-response assay. However, strains of AM fungi could differ in their ability to stimulate plant growth both in sterile soils [16] and in natural soils in the presence of indigenous AM fungi [17, 18]. The soil or substratum used needs to be the same or very similar to the one used in production [19], ensuring the fungi are tested at near field conditions. Furthermore, P fertilization could additionally affect both mycorrhizal colonization and effectiveness [1].

The present study was conducted to investigate the receptivity of a moderately acidic soil to non-indigenous AM fungi. Using samples from fields traditionally cropped with wheat in the southeastern of Argentinean Pampas, two experiments were set up to evaluate the capacity of some strains to develop within the soil, forming an effective AM symbiosis and increase growth. First, a receptivity trial was established at potential AM growth conditions (in sterile non-P amended soil and with a host plant highly mycotrophic and AM responsive) in order to select strains which could develop well in the soil and improve plant growth. Second, the development and the benefit expression of the most efficient non-indigenous AM strains were studied in inoculated wheat plants grown in a greenhouse under near field growth conditions (in P-fertilized soil with the native micro flora).

MATERIALS AND METHODS

Study Site and Arbuscular Mycorrhizal Fungi Determinations: Experiments were conducted at the INTA Balcarce Experimental Station, Buenos Aires, Argentina (37°45´S lat, 58°18´ W long ; 138 m a.s.l.). The climate of the region is humid-sub humid mesothermal. The annual mean temperature is 14.5°C and the annual average rainfall is 870 mm with 80% of rainfall during spring-summer (September-February). The soil is a moderately well drained Chernozemic loam (FAO soil classification), a Petrocalcic Paleudoll - series Balcarce, fine, mixed, thermic (USDA soil classification). It has a petrocalcic horizon at a depth of 1.2 m and a clay horizon at 33–74 cm depth. The topsoil (0-20 cm, Ap horizon) had the following properties: pH 5.7 (1:2.5 in water), organic matter (OM) 62 g kg^{-1} [20], Bray-P 8 mg kg^{-1} [21] and N-NO_3^- 15 mg kg^{-1} [22]. The soil is typical of the Argentinean Pampas, found on over 13 million ha and representing 43% of the Buenos Aires Province soils.

Mean soil temperature at 20 cm depth is 8.5°C in winter and 14.8°C in spring. The soil has a mycorrhizal potential of 0.70 (0.21–2.30) AM propagules kg dry $soil^{-1}$ (most probable number method, average of six replications, test plant: wheat;[23]).

Soil was collected from the site of a 10 year unfertilized wheat mono culture trial. Soil cores (each 0-20 cm depth and 5 cm diameter, total 5 kg) were randomly collected across transects from a field plot (10 ha agricultural of unfertilized wheat soil). Indigenous

AM spores were separated from the rhizosphere soil using a sequence of sieving, centrifugation and differential flotation on sucrose (60%) solutions according to the methodology described by Siverding [24]. Each spore type was mounted sequentially on microscope slides with water lactophenol, Polyvinyl-Lacto-Glycerol (PVLG) and Melzer's reagent for identification. Mycorrhizal identifications were based on current species descriptions and identification manuals with reference to taxonomic descriptions and images provided by web-sites of AM fungi [25, 26, 27, 28]. The morphological variables used for identification of spores were: occurrence of sporocarp and its shape, colour and size, occurrence of peridium and its characteristics, spore colour, size, surface ornamentation and wall structure. Indigenous mycorrhizal endophytes isolated were: *Acaulospora bireticulata* Rothwell and Trappe, *Acaulospora excavata* Ingleby and Walker, *Glomus etunicatum* Becker and Gerdemann, *Glomus microaggregatum* Koske, Gemma and Olexia, *Glomus mosseae* (Nicol. and Gerd.) Gerdeman and Trappe and *Gigaspora margarita* Becker and Hall.

Experiment 1. Receptivity Assay: Inoculation with Am Fungi in the Sterile Soil: Topsoil (5 kg) was collected as described above, ground (1-cm sieve) and steam tindalized twice (85°C , 2 h with 48-h between the two treatments) to kill the native microflora. After steaming the soil had pH 5.5, OM 52 g kg^{-1} and Bray-P 16.75 mg kg^{-1}. Soil was air-dried for 2 weeks in an isolated room and stored in black polyethylene bags at 10°C until use one month later. The experiment was established at the Laboratoire de Phytoparasitologie UMR INRA/Universite de Bourgogne BBCE-IPM, INRA-CMSE, Dijon, France. Onion (*Allium cepa* L. var. Topaze) was chosen as test plant because it is a highly mycotrophic species and respond quickly to mycorrhizal colonization [29]. Seeds of onion were surface disinfected in a 3.5% calcium hypochlorite solution for 10 min. After washing 5 times with sterile water 2 min each, the seeds were germinated in sterile vermiculite in a growth chamber in a constant environment room (18-h photoperiod provided by fluorescent lighting, 19/22°C mean temperature, 60/70% mean relative humidity, 320 μE m^{-2} s^{-1} irradiance). Ten days after emergence one plantlet was transplanted into an individual pot filled with 100-g of tindalized soil and was inoculated with AM fungi provided by the Banque Européene des Glomales (BEG). Inoculum had been produced in pots on leek (*Allium porrum* L.) growing in an acid sandy loam soil (pH 4.9, Olsen P 23 ppm) in a constant environment room (16-h photoperiod provided by fluorescent lighting, 19/22°C mean temperature, 60/70% mean relative humidity, 320 μE m^{-2} s^{-1} irradiance) during 6 months. Before inoculation mycorrhizal colonization in leeks roots was assessed [30]: only pot cultures with 50% root colonization by AM fungus and intense sporulation (up to 50 spores g $soil^{-1}$) were used as inoculum. A 10-g inoculums consisting of pieces of leek roots, external mycelium, spores and the adhering soil from pot cultures was placedin a hole beneath the onion seeds. Control pots received non-mycorrhizal leek roots.

The experiment was set up in a completely randomized design with five treatments of AM inoculation (non-inoculated –NI–; inoculated –I–: *Acaulospora longula* Spain and Schenck, isolate BEG8; *A. laevis* Gerdemann and Trappe, isolate BEG13; *Glomus claroideum* Schenck and Smith, isolate BEG31; and *G. clarum* Nicol and Schenck, isolate BEG142) and five replicate pots per treatment. Each strain used as inoculum was selected because it came from sites with some similar soil characteristics to the tested soil of Argentina but was not found among the native AM fungi. After inoculation, onion plants were grown in a growth chamber and watered daily with distilled water to maintain the soil humidity at water holding capacity (65% w/s). Plants received weekly 20-ml of Long Ashton nutrient solution minus P [31].

At 54 days after inoculation (DAI) plants were harvested and shoot fresh and dry matter (SFM, SDM, respectively) were measured. The entire root material was washed out free of soil, collected on sieve (0.5 mm) and root fresh matter (RFM) was recorded after roots were uniformly blotted with a filter paper to absorb excess moisture. Immediately, roots of each treatment were cut into 1-cm pieces, thoroughly mixed and divided into two batches: one was used for histochemical staining to quantify the alkaline phosphatase (ALP) activity according to the procedure described by Tisserant *et al*. [32]: roots were covered for 2-h with ice-cold 10% sorbitol –Tris/acid buffer (0.05 M, pH 9.2) solution and then stained at room temperature overnight after addition of 20 ml Tris/citric acid buffer (0.05 M, pH 9.2) containing 1 mg ml^{-1} α-naphtyl acid phosphate (Sigma), 1 mg ml^{-1} Fast Blue salt, 0.05% $MgCl_2$ and 0.05% $MnCl_2$. The remaining roots were stained by the classical non-vital trypan blue (TB) staining procedure [33]: roots were cleared with KOH (10%, 30 min, 90°C), acidified with HCl (0.1 N, 2 min, room temperature) and stained with TB (0.05%, 5 min, 100°C). Mycorrhizal infection was assessed by microscopic

examination (40X) and estimated as percentage of root colonized by AM fungus (M%) and percentage of roots colonized by arbuscules (A%), according to the Trouvelot *et al*. [30] method.

Mycorrhizal responsiveness (MR) was calculated from Eq. 1 using the individual total plant SDM of inoculated (I) plants and mean SDM of non-inoculated (NI) plants [34]:

$$MR = \frac{SDM(I) - \text{mean SDM (NI)}}{\text{Mean SDM (NI)}} \times 100 \quad (1)$$

Experiment II. Inoculation of Wheat with Selected AM Fungal Strains in the Presence of Native Mycorrhizal Fungi and P Fertilization: This experiment was undertaken from August to November at the INTA Balcarce Experimental Station, Buenos Aires, Argentina. The topsoil (0-20 cm, Ap horizon, 120 kg) and subsoil (20-40 cm, B_1 horizon, 100 kg) were separately collected from the site of Experiment 1, as described above, and ground to pass a 1 cm sieve. The topsoil had pH 5.7, OM 62 g kg^{-1}, Bray-P 6.5 mg kg^{-1} and N-NO_3^- 15 mg kg^{-1}, the subsoil had pH 5.9, OM 37 g kg^{-1}, Bray-P 4.0 mg kg^{-1} and N-NO_3^- 4 mg kg^{-1}.

Experimental units consisted on plastic pots (diameter 10 cm, height 30 cm) containing 3 kg of soil each. To simulate the soil profile, 1 kg of the subsoil was placed at the bottom (20-30 cm) of the pots and 2 kg of the topsoil was placed at top (0-20 cm) of the pots. Seeds of wheat (*Triticum aestivum* L., cv. ProINTA federal) were surface disinfected (7% calcium hypochlorite solution, 30 min, then washed 5 times with sterile distilled water 2 min each), cold-treated (4°C, 2 days), germinated and maintained in a constant environment room (12 h photoperiod provided by fluorescent lighting, 21/24°C mean temperature, 55/65% mean relative humidity, 320 µE m^{-2} s^{-1} irradiance) until 3 days after emergence. Two germinated wheat seedlings were transplanted into each pot.

The experiment was set up in a completely randomized design with 2 rates of P (0 and 8 mg kg $soil^{-1}$: -P and +P, respectively) and four treatments of AM inoculation (non-inoculated –NI–, inoculated –I–: *A. longula*, *G. claroideum* and *G. clarum*) in factorial arrangement with three replications. Inoculum were the same as used in Experiment 1 and provided by the BEG one month before the experiment and stored at 4°C in black polyethylene bags until use.Inoculum consisted of pieces of leek roots (with at least 50 % M), external mycelium, spores (at least 50 spores g $soil^{-1}$), and the adhering soil from pot cultures. A total of 30 g of inoculum was used beneath the onion seeds dispersed as follows: 10 g at 25 cm depth, 10 g at 15 cm depth, and 10 g at 5 cm depth. Control pots (NI) received non-mycorrhizal roots of leek and the adhering soil from pot cultures. In the P fertilized treatments, each pot was amended at sowing with 120 mg commercial triple-calcium superphosphate as a nutrient aqueous solution (at a rate equivalent to 8 mg P kg $soil^{-1}$ or 20 kg P ha^{-1}). All pots received nitrogen at sowing. Commercial urea was applied in aqueous solution of 334 mg urea pot^{-1} (at a rate equivalent to 50 mg N kg $soil^{-1}$ or 125 kg N ha^{-1}). Phosphorus and N fertilization corresponded to moderate-low rates as used by Argentinean farmers at improving wheat production. Plants were grown in a greenhouse (30-14°C mean temperature day-night, respectively, 40-100% mean relative humidity day-night, respectively, 7.8±1.1 MJ/m^2 day mean daily solar radiation at the topthe plants) and daily watered with distilled water to maintain the soil at water holding capacity (65% w/s).

After 79 DAI plants were in ripening phase ([35]; code 85) and were harvested. The aerial part of plants were cut and shoot dry matter (SDM: leaf and shoot) and grain dry matter (GDM) were measured. Each plant material was separately ground (< 2 mm sieve) and P concentration in each sample was measured after a nitric-perchloric acid digestionusing a colorimetric approach with ascorbic acid [36]. Total shoot P concentration (Spc) was calculated by averaging by weight the P concentration of shoot and grains. The P uptake of shoot and grains were calculated by multiplying the SDM or GDM by the P concentration of each part, respectively. Total shoot P uptake was calculated by adding the P uptake of shoot and grains. Pots were opened and the soil-root system was divided in two fractions (0-20 cm and 20-30 cm depth from the top of pots) and separately analysed. Sieved soil samples (1 cm) free from roots, were taken from each soil-pot fraction to determine available soil P, which was extracted according to Bray and Kurtz [21]. Determination of RFM on each fraction was the same as described for *Experiment I*. Afterwards, roots were cut (1 cm), thoroughly mixed and divided into two batches: one (50% of RFM) was used to measure root dry matter (RDM). Remaining fresh roots were used to determine M% and A% after TB staining as described for *Experiment I*.

Mycorrhizal responsiveness in total shoot P uptake (Eq. 2), SDM, GDM and in P uptake of grains (each

similarly as described in Eq. 2) were separately calculated for fertilized and unfertilized plants:

$$\text{MR P uptake} = \frac{\text{Total shoot P uptake (I)} - \text{mean total shoot P uptale (NI)}}{\text{mean total shoot P uptake (NI)}} \times 100 \quad (2)$$

For each parameter, MR was calculated using the individual parameter of inoculated plants (I treatments: colonized with the indigenous plus non-indigenous AM fungi) and the mean parameter of non-inoculated (NI treatments: plants colonized only with the indigenous AM fungi).

Statistical Analysis: The data were subjected to Analysis of Variance using the Statistical Analysis Systems (SAS) package [37]. Normality of data was tested by the Shapiro and Wilks's Test. As the null hypothesis (H_0= data are normal) was not refused, all data presented are means of untransformed values. Means were compared by using the least significant difference (l.s.d.) procedure at the 0.05 probability level. There was an evident vertical distribution of mycorrhizal colonization (inoculated or native mycorrhizal) throughout the root depth-profile of wheat plants. Highest soil P content, RDM and AM colonization were found in the upper 20 cm depth of pots. Additionally, significant effects of treatments were was determined at 0-20 cm of pots,but no treatment effects were found below 20 cm depth. Thus, we present soil-root parameter results of Experiment 2 only at 0-20 cm depth of pots.

RESULTS AND DISCUSSION

Experiment I. Receptivity Assay: Inoculation with AM Fungi in the Sterile Soil: All AM fungal isolates were infective in the moderately acidic Argentinean soil tested (Table 1) and clear differences in mycorrhizal development occurred among isolates. As expected, neither mycorrhizal formation nor ALP activities were present in the non-inoculated plants. Tindalization of soil ensured the elimination of indigenous AM fungi. It has long been recognized that the percentage of root colonized using no vital stains can result in an overestimation of functional colonization, as dead and metabolically inactive fungi are included in the data collected. A wide range of mycorrhizal colonization (0-91%) and ALP activity (0-73%) were found. ALP activity was always significantly ($P<0.05$) lower than fungal tissue TB-stained. Zhao *et al*. [38] also reported higher mycorrhizal colonization values with TB than ALP procedures. Kough and Gianinazzi-Pearson [39] indicated that the TB staining technique overestimates mycorrhizal activity, because not all the AM fungal tissue revealed by the non-vital staining is necessarily physiologically active. However, van Aarle *et al*. [40] mentioned that when the majority of the structures observed are metabolically active, TB staining will not overestimate colonization in relation to ALP. The effectiveness of AM fungi has been related to the extent of fungal colonization and the metabolic activity of intra- or extraradical fungal structures [41]. For the quantification of AM fungi activity in plants, the estimation of the total fungal biomass in the roots using non-vital staining like TB [33] could be insufficient for assessing mycorrhizal activity [38]. The quantification of the fungal enzyme ALP has been shown to indicate active mycorrhizal infection [42] and could be a useful marker to analyse the efficiency of AM infections [32, 38].

The rate of colonization and arbuscule content as revealed both by TB staining and ALP activity were, in general, significantly and differentially highest in roots inoculated with *G. clarum* as with *A. longula* (Table 1). Plants inoculated with *A. laevis* produced the lowest levels of AM colonization as revealed by TB and ALP activity staining. Inoculation with *G. claroideum* produced intermediate colonization and arbuscules as revealed by the both staining used methods.

Formation of AM significantly enhanced differently RFM, SDM and the growth response of plants (Table 1). Colonization with *G. claroideum* produced the highest root and shoot mass and thus the highest MR was obtained. Although inoculation with *A. longula* produced intermediate shoot and root mass, it obtained as high an MR as *G. claroideum*. *Glomus clarum* produced intermediate to low MR; no growth benefit was determined after the inoculation with the *A. laevis* strain. Differences in root development among treatments could have compensated for differences in percentage of root colonization. If the amount of root production is associated with the percentage of arbuscules that are ALP active, plants inoculated with *G. claroideum* resulted in higher root mass with ALP active arbuscules (294 mg plant^{-1}) than plants that showed the highest colonization when inoculated with *G. clarum* (171 mg plant^{-1}). Arbuscules are believed to be the main site of nutrient exchange between the host plant and the AM fungi [43]. Our results show that *G. claroideum* formed more ALP

Table 1: Expt 1: Effect of mycorrhizal inoculation on root mycorrhizal colonization (M) and percentage of arbuscules (A) estimated by staining with trypan blue and for alkaline phosphatase (ALP) activity; shoot and root fresh mass (SFM, RFM, respectively), shoot dry mass (SDM) and mycorrhizal responsiveness (MR) of onion test plants grown in a steam-pasteurized Argentinean soil

	Staining procedure							
	Trypan blue		ALP					
	M	A	M	A	SFM	RFM	SDM	MR
Treatment	(%)				(mg plant^{-1})			(%)
NI	0.0d	0.0e	0.0d	0.0d	170c	70b	30d	--
A. laevis	17.1c	5.0d	6.0c	3.1c	190c	60b	30d	0c
A. longula	79.3ab	69.1b	62.3ab	52.2ab	960b	310b	224b	647a
G. claroideum	63.1b	53.8c	47.4b	42.4b	1510a	700a	294a	880a
G. clarum	91.1a	85.7a	73.1a	61.3a	860b	280b	132c	342b
l.s.d. (P <0.05)	16.0	10.0	13.0	15.0	353	248	69	259

Treatments: non-inoculated, NI; inoculated, *A. laevis*, *A. longula*, *G. claroideum* and *G. clarum*, Different letters in columns indicate significant differences at P<0.05 among treatments by using analysis of variance (ANOVA) and mean separation (LSD) [38]

Table 2: Expt 2: Effect of P fertilization and mycorrhizal inoculation mycorrhizal colonization (M), percentage of arbuscules (A), shoot dry matter (SDM), total shoot P uptake (SPuptake), grain dry matter (GDM), grain P uptake (GPuptake) and root dry matter (RDM) of spring wheat plants grown in an Argentinean soil in the presence of native AM fungi

Treatment		M	A	SDM	SPuptake	GDM	GPuptake	RDM
Phosphorus	Inoculation	(%)				(mg plant^{-1})		
- P	NI	17.5b	10.1ab	2715d	3.12e	1152e	2.80c	246e
	A. longula	17.0b	9.4ab	3087d	3.67de	1327de	3.32c	316e
	G. claroideum	22.0a	12.3a	3692c	4.23cd	1483dc	3.62bc	450bc
	G. clarum	19.0ab	10.2ab	3128d	3.48de	1242e	3.10c	335de
+ P	NI	9.0cd	3.2b	4058bc	4.91bc	1633bc	4.33ab	553ab
	A. longula	12.0c	9.8ab	4725a	5.47ab	1910a	4.76a	568a
	G. claroideum	15.5bc	10.9ab	4413ab	5.86a	1777ab	4.97a	569a
	G. clarum	5.0d	2.2b	3983bc	4.97bc	1545c	4.33ab	437cd
l.s.d. (P<0.05)		4	4	461	0.818	188	0.972	111

Treatments: non fertilized, -P; P fertilized soil, +P; non inoculated, NI; inoculated, *A. longula*, *G. claroideum* and *G. clarum*

active mycorrhizal tissue than *G. clarum* and thus appeared to have higher mycorrhizal plant benefit.

Non-significant correlations were found between MR and colonization as revealed by TB and ALP activity staining (data not shown). Van Aarle *et al*. [44] also reported that the proportion of ALP active arbuscules, although they seemed to increase with growth, were not significantly correlated with shoot weight ofonion. In our experiment, the *G. clarum* and *A. longula* strains consistently formed the highest percentages of root colonization and arbuscular ALP activity. However, *G. clarum* did not significantly increase onion plants growth in comparison to the isolates that also formed high colonization (*A. longula* and *G. claroideum*). The lack of a significant relationship between MR and colonization confirms that, when assessing ineffective strains (as *G. clarum*), the extent of root colonization is a poor indicator of mycorrhizal growth effects [45].

Acaulospora longula was one of the strains which formed the highest proportion of metabolically ALP active colonization and also was an effective strain at improving growth of onion in the moderately acidic Argentinean soil tested. *Glomus claroideum* had intermediate ALP active mycorrhiza, and was the most efficient strain at improving growth. *Glomus clarum* showed the highest percentage of root colonized and ALP activity, but this did not correlate with the greatest increase in plant growth. Thus, although *G. clarum* was highly infective, it was not an efficient strain to improve plant growth in the testedsoil. *Acaulospora laevis* was not effective either at colonizing roots or at increasing plant growth

Experiment II. Inoculation of Wheat with Selected AM Fungal Strains in Presence of Native Mycorrhizal Fungi and P Fertilization: In this experiment, AM strains that proved to be efficient at improving plant growth (such as *G. claroideum* and *A. longula*) and/or highly infective (such as *G. clarum*) in Experiment I were selected tobe tested for improving wheat growth in pots

under near field-growth conditions. AM fungi- and P amended).

Phosphorus fertilization increased the available soil P content (mean value of soil P content: 5.80 and 9.57 mg kg soil^{-1} for – P and + P treatments, respectively; l.s.d. 2.33) and non significant differences among inoculation treatments were found for the availability of soil P. Mycorrhizal colonization was in the range 5-22% (Table 2). Zhu *et al*. [46] reported that AM colonization of different wheat cultivars growing in pots until 7 weeks (flowering) varied from 16% to37%. In this experiment, plants were grown until ripenin and at ripening colonization decreases because seeds are the main sink of carbohydrates, acceptable levels of colonization were obtained. Mycorrhizal colonization was in general lower in P supplied (+P) plants compared with the nil P (-P) treatments (Table 2). The negative effect of P fertilization on AM colonization was evident and is well documented in wheat [13, 47, 15]. At this experiment, however, the effect of P fertilization on AM colonization differed with the strains. In growth conditions without P, the AM colonization was highest in plants inoculated with *G. claroideum*, followed by plants inoculated with *G. clarum* and the NI ones (colonized only with the native AM population). With added P, *G. claroideum* showed as much AM as plants inoculated with *A. longula*; plants that received *G. clarum* had the lowest AM colonization. Also, P fertilization did not affect A% in either *A. longula* and *G. claroideum* inoculated treatments. Thus, plants inoculated with one of each the non-indigenous strain seemed to be less affected by the P supply in comparison with plants inoculated with *G. clarum* and with NI ones. This indicates that some AM fungi are more sensitive to P fertilization than others as reported by Rubio *et al*. [15].

Plants that were both fertilized and inoculated had a greater SDM, GDM, P uptake and RDM. With added P, the highest SDM and GDM were obtained after the inoculation with the A. longula strain followed by the *G. claroideum*. However, the latter strain produced SDM thatwas not significantly different than that produced by *G. clarum* or in the NI treatment. The highest shoot P uptake was obtained with added P after the inoculation with *G. claroideum*strain followed by A. longula. The highest P uptake of grains was obtained after inoculation with the A. longula and the *G. claroideum* strains, but they were not different than produced by the *G. clarum* or the NI treatment. Also Zhu *et al*. [46] found that AM colonization with *Glomus intraradices* increased tissue P concentrations both of old and modern wheat growing in pots. However, in order to destroy indigenous mycorrhizal fungi they grown wheat in autoclaved soil/sand mixture. At our experiment, P uptake was improved with inoculated plus indigenous AM fungi. The RDM was not significantly different among plants non-inoculated or inoculated with *G. claroideum* or *A. longula*. With nil P, *G. claroideum* also produced the highest SDM in relation to the other treatments, inoculated or not. The highest shoot P uptake and GDM were obtained after inoculation with *G. claroideum* as with the *A. longula* strain. The highest RDM was obtained in plants inoculated with the *G. claroideum*.

Zhu *et al*. [46] reported that mycorrhizal colonization depressed plant growth both in old and modern wheat cultivars inoculated with *Glomus intraradices* growing in pots with an unfertilized soil/sand mixture with $NaHCO_3$-extractable P of 9.4 mg P kg^{-1}, probably because to low irradiance of growth conditions. At our experiment, positives MR were obtained, particularly at low soil P availability. Mycorrhizal growth responses were, in general, two-fold lower (P<0.05) with added P in comparison to the nil P treatment (Fig. 1). Similar negative

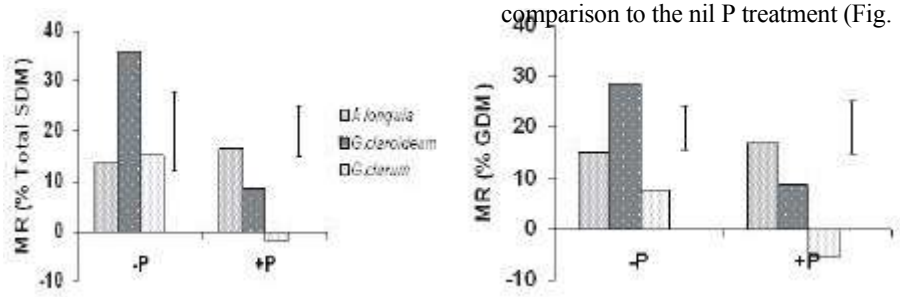

Fig. 1: Expt 2: Effects of P fertilization and mycorrhizal inoculation on mycorrhizal responsiveness (MR) in total shoot dry matter (SDM), grain dry matter (GDM), total shoot P uptake and grains P uptake of spring wheat plants grown in an Argentinean soil in presence of native AM fungi.
Fertilization treatments: non fertilized, -P; fertilized, +P Inoculation treatments: *A. longula*, *G. claroideum*, *G. clarum*
Error bars denote l.s.d. at P<0.05

responses to P application by plants grown in AM inoculated soils were noted by Pfleger and Linderman [1], Mohammad *et al*. [47] and Rubio et al. [15]. These results clearly showed the greatest mycorrhizal benefit in conditions without P supply or with low available soil P. Abbott and Robson [48] reported that all AM fungi do not contribute equally to nutrient uptake and plant growth. In this experiment, plants inoculated with *G. claroideum* had the highest MR in comparison to the *A. longula* or the *G. clarum* strains, particularly with nil P. With added P, the highest MR was obtained after inoculation with *A. longula* or *G. claroideum*. Plants inoculated with *G. clarum* produced, in general, the poorest growth, which was equivalent to the NI treatment.

Differences in the ability of AM isolates to stimulate wheat growth have been reported by Talukdar and Germida [12]. However, they showed that inoculationwith *G. clarum* isolate NT4 enhanced growth and yield of wheat in an autoclaved soil/sand mix of pH 7.6. Later, Xavier and Germida [13] showed that *G. clarum* NT4 increased the yield of wheat in a non-sterile soil of pH 7.2 and OM 30 g kg^{-1} which contained AM fungi and other indigenous microorganisms. In this case, the growth response varied among cultivars and soil P supply treatments. We found that inoculation with the isolate BEG 142 of *G. clarum* was ineffective at improving wheat growth when inoculated in the moderately acidic tested soil (with and without P-fertilization) in the presence of indigenous AM fungi and other microorganisms. It is likely that differences in soil characteristics or the aggressiveness of indigenous soil micro organisms could account for the variable response to the inoculation with *G. clarum*. On the other hand, differences in AM symbiotic efficiency have been attributed to the spread of root colonization, the production of external mycelium and to the amount of arbuscular tissue present in the colonized roots [39]. Although in this experiment mycorrhizal colonization values were low because it was determined at plant maturity, arbuscule contents of wheat, particularly with added P, were higher in plants inoculated with *A. longula* or with *G. claroideum* than in those only with indigenous AM population (NI treatments) or the *G. clarum* fungus were developed. It is probably that high AM colonization formed for both strains have contributed to the P uptake (as showed by the greater MR) and to the production of grain dry matter and thus they were the most effective tested strains to improve wheat growth.

CONCLUSIONS

The combination of the two experiments allowed selecting a non-indigenous AM inocula that could develop well in the soil and increase wheat plant growth. van Aarle *et al*. [40] reported that although ALP activity was observed in intercellular root colonization at *Allium porrum* plants, any growth responses to mycorrhizal colonization after inoculation with *G. intraradices* were found. They related the lack of MR to the fact that the experiment was carried out in a glasshouse in winter with no supplementary light, conditions that have frequently been shown to reduce or eliminate mycorrhizal growth responses in *Allium porrum* and other hosts. At our experiment, clear mycorrhizal responses were obtained both for onion and wheat plants. For onion, the controlled light conditions, unfertilized and sterile soil permitted to select the better AM strains after removing any possible inhibitor effect on mycorrhizal fungus growth. The test plant used was clearly appropriate for selection of strains at the receptivity trials because it quickly formed mycorrhizal colonization levels that where in some cases three-fold higher in comparison to those obtained for the wheat plants. Although the growth responses of onion plants where in general one order of magnitude higher than the obtained for wheat, clear MR were obtained after growing inoculated wheat plants in glasshouse with no supplementary light. Thus, the combination of TB and ALP staining procedures plus the MR of onion plants permitted to select strains potentially efficient to be inoculated in the soil. Furthermore, result sobtained in 'potential' growth conditions for the development and the benefit of expression of the strains in the soil were validated for wheat plants under near-field growth conditions (although in pots in greenhouse, in the P-fertilized soil with the indigenous AM fungi and other micro organisms).

The isolate BEG 31 of *G. claroideum* formed intermediate AM colonization in the sterile soil, an ALP active mycorrhiza and was the most effective strain at improving growth of the test plant. Then, it colonized wheat and had highest P uptake of shoot and grain and produced, in general, highest mycorrhizal growth benefit when was grown with the native AM population and fertilized at a moderate P dose. The isolate BEG 8 of *A. longula* produced intermediate mycorrhizal root colonization when developed in the sterile soil and

produced the high growth response of the test plant. Then, the strain colonized wheat roots and produced intermediate-high growth response to mycorrhiza, particularly with added P. The isolate BEG 142 of *G. clarum* was the most infective strain when developed within the sterile soil. However did not produce the highest growth response of the test plant nor produced highest MR of wheat plants when the strain developed with the indigenous AM fungi. Thus; it is unlikely probably that *G. clarum* is an efficient strain to inoculate wheat under field conditions at the Argentinean Pampas. The soil disinfected was not receptive to the isolate BEG 13 of *A. laevis* since this strain was not effective at colonizing roots or at increasing plant growth.

In summary, shoot dry matter of wheat substantially increased upon P fertilization, but it was only marginally increased by inoculation with some isolates. It has been reported that wheat is not highly dependent on mycorrhiza [10, 11, 46]. In general, plants with finely branched root systems like wheat [49] depend less on mycorrhizae for growth becausethey more efficiently explore the soil P than plants with coarsely branched roots and with few or no root hairs [11, 13]. Mycorrhizal benefit was lower with P fertilization in comparison with nil P treatments. When wheat was grown in the soil containing indigenous AM fungi and others microorganisms with moderate P fertilization, inoculation with *A.longula* and *G. claroideum* formed mycorrhizal symbiosis which significantly increased growth, P uptake and grain production. The introduced AM strains were more effective at increasing wheat growth and P uptake than the native AM fungi. Further field experiments are required to determine if the inoculation with the non-indigenous AM fungi at the Argentinean pampas soils could enhance acceptable wheat yields with a reduction of crop costs. Additionally, an understanding of competitive interactions among the introduced AM fungi to the other indigenous soil microbes is required to predict the likely outcome of management practices, including inoculation, on colonization and growth benefit of wheat.

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ABBREVIATIONS

AM, arbuscular mycorrhizal; A%, percentage of arbuscules; ALP, alkaline phosphatase activity; DAI, days after inoculation; GDM, grain dry matter; GPuptake, grain phosphorus uptake M%, mycorrhizal colonization; MR, mycorrhizal responsiveness; N, nitrogen; NI, non-inoculated; OM, Organic matter; P, phosphorus; -P, nil P; +P, added P; SFM, shoot fresh matter; SDM, shoot dry matter; SPuptake, shoot P uptake; RDM, root dry matter; RFM, root fresh matter; TB, trypan blue