Arbuscular mycorrhiza enhances nutrient uptake in chickpea

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ABSTRACT

Arbuscular mycorrhiza fungi (AMF) colonize roots of host plants and promote plant growth due to improved uptake of nutrients. While the effects on P uptake are well known, the relevance of AMF for the uptake of other nutrients is less investigated. In the present paper we studied contents of N, P, K, Ca, Mg, Fe, Mn, Cu, and Zn in the legume chickpea in pot experiments during two seasons. Beside the control, the following treatment combinations: (i) the inoculation with the commercial AMF product 'Symbivit®'; (ii) soil sterilization before inoculation, and (iii) mineral nitrogen application. A moderate level of AMF colonization (18–55% of roots), enhanced the nutrient uptake of chickpea. With P, Mn, and in 2006 also with K, Cu, and Fe the nutrient concentrations were also elevated, even along with a simultaneous increase in plant biomass. Soil sterilization or fertilization with N showed no significant effect on nutrient uptake and biomass production.

Keywords: AMF inoculation; nutrient concentration; nutrient acquisition; legume; soil sterilization

Arbuscular mycorrhiza fungi (AMF) colonize roots of host plants and promote plant growth, which is generally attributed to the improved uptake of nutrients with particular emphasis on P nutrition (Hirata et al. 1988, Smith and Read 2008). Recently, we reported that AMF colonization fostered the growth of chickpea up to +43% of total dry matter (Farzaneh et al. 2009). AMF effects on nutrient uptake in chickpea were reported only by Alloush et al. (2000), Zaidi et al. (2003), and Akhtar and Siddiqui (2007). In these studies, nutrient concentrations in plant tissue were often not affected or even reduced.

Effects of mycorrhizal symbiosis may vary with the soil nutrient status (Gamper et al. 2004). Growth and N acquisition of mycorrhizal plants benefit from N fertilization depending on the N availability from the soil (Jia et al. 2004, Azcón et al. 2008). In addition, there is evidence that genetic variability in the fungal partner influences N acquisition (Vázquez et al. 2001).

The mycorrhizal effects can also be affected by soil sterilization, which is often used before inoculation to optimize infection conditions for experimental AMF strains. Many studies demonstrated beneficial effects of indigenous and inoculated AMF on legumes (Habte and Aziz 1985, Chalk et al. 2006). We observed no effect of soil sterilization on the growth enhancement due to AMF inoculation (Farzaneh et al. 2009), but other studies indicated that AMF inoculation increased dry matter accumulation and nutrient uptake compared with indigenous AMF communities (Biró et al. 2000).

The objectives of the present paper were (i) to evaluate the effect of AMF inoculation on macro and micro nutrient concentrations and uptake of chickpea in sterilized soil and (ii) to determine the importance of indigenous soil microorganisms for nutrient acquisition in non-sterilized soil. In order to study AMF effects on N nutrition more in detail, AMF inoculation was tested in combination with or without an application of mineral fertilizer N.

MATERIAL AND METHODS

Experiments were carried out in Mitscherlich pots during spring and summer 2006 and 2007 (Table 1) in a fence house in Vienna, Austria. The experiments were in a randomized complete block design with five replications, including the following factors:

– Year (2006 or 2007)
– AMF inoculation (M+ or M–, i.e. with or without inoculum)
– Soil sterilization (S+ or S–, i.e. with or without initial sterilization of soil, the latter only in 2007)
– Nitrogen nutrition (N+ or N–, i.e. with or without mineral fertilizer)

Chickpea (commercial seeds of an unidentified Kabuli genotype) were sown in a mixture (1:1) of sterilized soil and sand and manually thinned out to three plants per pot.

The soil was a Chernozem topsoil (texture silty loam) derived from the experimental farm Gross-Enzersdorf of BOKU University. The soil-sand substrate was analyzed for nutrient availability (Table 1) and subsequently sterilized (105°C, 24 h) before sowing. Additional to the sterilization treatment, only in 2007 we studied the effects of omitting soil sterilization.

Inoculation of the AMF treatments was done by adding the commercial AMF inoculum 'Symbivit®' (Symbio-m, s.r.o., Lanškroun, Czech republic) below seeds at a rate of 5 g for a group of three seeds which were later thinned to one plantlet.

Generally, no rhizobial strains suitable for infection of chickpea were expected to be in the non-sterilized field soil, because no chickpea had been grown there before. This was confirmed by the complete absence of nodules on chickpea roots in all samples either with or without sterilization. Thus chickpea N nutrition was based only on soil mineral N. Pots with N application (N+) received an amount of 314 mg N/pot (equivalent to 100 kg N/ha) as a calcium ammonium nitrate solution one week after emergence. Tap water (4 mg NO_3/L) was supplied during the vegetation period daily if necessary to about 90% of soil water capacity in order to avoid any drought stress.

Plants were harvested at physiological maturity and were divided into the plant tissue fractions roots, straw and pods. Root samples were washed carefully with tap water above a sieve (250 µm) in order to omit root losses. Then samples were dried (105°C, 24 h) and the tissues were milled (1 mm). AMF root colonization was determined as described by Farzaneh et al. (2009). Chemical analyses were performed separately for the plant fractions, and nutrient concentrations and uptake were recalculated for complete plants based on the dry matter shares.

The concentration of nitrogen in the samples was determined using an element analyzer (LECO CN, St. Joseph, USA). For other elements, dried samples (ca. 500 mg) were digested in acid (Nabrzyski and Gajewska 1998) and contents of K, Ca, Mg, Fe, Mn, Cu, and Zn in the digest were measured using atomic absorption spectrometry (Varian SpektrAA 300, Vienna, Austria) (Beaty and Kerber 1993). P was analyzed by a spectrophotometer (Varian DMS 200) (Cavell 1955).

The statistical analysis was done with all experimental data by analysis of variance using the procedure MIXED of the SAS system. The statistical model assumed a random block effect and fixed effects of year, inoculation, sterilization and nitrogen fertilization. With significant ($P < 0.05$) factorial effects, the *t*-test was used to calculate the least significant differences (*LSD*) for comparisons of means.

RESULTS

ANOVA proved with high consistence either main effects of AMF inoculation or interactions of AMF with year or soil sterilization. Effects of nitrogen fertilization, which were found rarely and at low significance levels, are not important for our objectives.

The inoculation with AMF was successful, because all inoculated plant samples were substantially colonized (Table 2). Without sterilization, the soil obviously contained indigenous populations that were able to colonize chickpea. Yet, inoculation increased colonization also in non-sterilized soil.

Table 1. Characteristics of pot experiments (one bulk soil sample per year)

Growing season	Unit	2006	2007
pH value ($CaCl_2$)	–	7.5	7.5
$N0_3$-N	mg/kg	5.6	10.7
	kg/ha	25	48
Plant available P (CAL)	mg/kg	106	138
Plant available K (CAL)	mg/kg	191	248
Plant available Mg ($CaCl_2$)	mg/kg	94	115
Date of sowing	–	25 and 26 April	23 and 24 April
Harvest date at maturity	–	25–27 July	7 August

Table 2. Percentage of AMF colonized roots and total chickpea biomass

Year	Soil sterilization	AMF colonization (%)		Total biomass (g/pot)	
		M+	M–	M+	M–
2006	with sterilization	54.5 (3.7)	0 (0)	11.92 (0.48)	11.76 (0.64)
2007		24.9 (6.3)	0 (0)	11.77 (1.19)	8.25 (1.12)
2007	without sterilization	44.1 (7.1)	17.8 (3.9)	9.05 (0.83)	6.99 (0.59)

Means and their standard errors (SEM, in brackets). Treatments without (M–) or with (M+) AMF inoculation and without (only in 2007) or with soil sterilization (n = 10)

After soil sterilization without AMF inoculation no colonized roots were found at all.

Mycorrhiza increased total dry matter in both years (2006–2007), but this enhancing effect was significant only in 2007 (Table 2). This resulted from significant differences in all plant tissue fractions between inoculated and non-inoculated plants (data not shown). The positive inoculation effect can be confirmed also for the non-sterilized soil.

Mycorrhiza effects. It can be generalized across 2006 and 2007 (main effect inoculation) that the P concentration was higher and the N concentration was lower in AMF chickpea than in control plants (Table 3). Only Mg and Zn concentrations were unaffected by mycorrhizal inoculation.

The effect of AMF inoculation on the concentrations of K, Ca, Fe, Mn, and Cu partly depended on the year. AMF induced higher Mn concentration in both years but this effect was more pronounced in 2007. Mycorrhizal inoculation decreased the Ca concentration in 2006 significantly while in 2007 it was slightly increased. Only in 2006 mycorrhizal inoculation resulted in higher K, Cu and Fe concentrations. The high absolute level of Fe concentration was due to extraordinary high Fe concentrations in the roots (data not shown).

Reflecting also differences in dry matter, the uptake of P, K, Mg, Fe, Mn, Zn, and Cu per pot in both years and Ca in 2007 increased with AMF inoculation (Table 4). The increment of Mn uptake was substantially higher in 2007.

Comparison of sterilized vs. non-sterilized soil in 2007. AMF colonization resulted in a significantly higher Mn concentration in both soils, but with a stronger effect in sterilized soil (Table 5). In non-sterilized controls, there was no clear difference in P concentration due to AMF inoculation. In contrast, in sterilized soil, the P concentration of plants with AMF inoculation was significantly higher than of those without AMF. AMF effects on N, K, Ca, Fe, Zn, and Cu concentrations were not affected by soil sterilization but followed the trends described above (cf. Table 3).

There were no significant interactions in nutrient uptake between AMF inoculation and sterilization of soil except for Mn: The positive effect of inoculation on the uptake of Mn was more pronounced in sterilized soil (Table 5).

Table 3. Nutrient concentrations in total chickpea biomass as affected by treatments without (M–) or with (M+) AMF inoculation in interaction with year (n = 10 or 20)

		Nutrient element								
		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		(g/kg)					(mg/kg)			
M+	2006	24.26	1.55	14.71	16.42	3.11	2101	107.81	39.59	10.74
	2007			13.02	20.12	3.42	2051	166.62	35.41	11.53
M–	2006	27.38	0.96	11.59	22.39	3.09	891	85.52	38.61	9.06
	2007			12.77	18.60	3.19	1944	120.31	34.94	11.81
$LSD_{0.05}$		2.14	0.12	1.17	2.20	n.s.	628	14.32	n.s.	0.88

n.s. – not significant

Table 4. Nutrient uptake in total chickpea biomass as affected by treatments without (M–) or with (M+) AMF inoculation in interaction with year (n = 10 or 20)

		Nutrient element								
		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
		(mg/pot)					(µg/pot)			
M+	2006	218.1	18.51	163.1	202.7	38.38	24296	1335.9	440.0	130.4
	2007	334.4			228.0			1879.8		
M–	2006	221.8	9.16	112.0	257.4	29.63	12597	987.8	350.0	96.4
	2007	263.2			145.4			917.1		
$LSD_{0.05}$		n.s.	2.59	15.7	57.8	5.45	5419	345.8	50.6	17.6

n.s. – not significant

DISCUSSION

Mycorrhiza effects on chickpea. Positive effects of AMF on nutrient acquisition were explained by increased root lengths, coupled with the extra soil volume that can be exploited by hyphae (Daft 1991). As reported previously, our study confirms a positive effect on root growth after successful inoculation of chickpea with AMF, yet the growth promotion on shoots was even stronger (Farzaneh et al. 2009).

In the present study, AMF colonization significantly reduced the N concentrations compared to the control irrespective of the N supply level. This might be attributed to a dilution effect due to higher dry matter production in the AMF treatment. This result is in accordance with other studies (Hirata et al. 1988, Gavito et al. 2000). Note that interactions of AMF with biological N fixation were not included in our study.

While no significant variation of the Zn concentration was observed after mycorrhizal inoculation, we found a positive impact of AMF colonization on the P, K, Fe, Mn, and Cu concentrations, partly depending on the year. In 2007 a similar effect was also shown for Mg when including non-sterilized soil. These increases in concentration are even more remarkable due to the simultaneous increase in plant biomass. The experimental soil in our study showed moderate (2006) to high (2007) P, K, and Mg concentrations. In other experiments on nutrient acquisition of chickpea (Hirata et al. 1988) and pea (Gavito et al. 2000) under sterile soil conditions and high soil P levels, P and K concentrations were not improved by AMF inoculation.

Our analyses revealed a negative effect of AMF colonization on Ca concentration compared to non-colonized plants in one year. Several previous studies showed inconsistent results regarding Ca concentrations in different species due to mycorrhizal colonization in combination with various soil pH or initial P values (Clark 1997, Alloush et al. 2000, Bagayoko et al. 2000). Marschner and Dell (1994) suggested that mycorrhizal plants might try to maintain low plant Ca concentrations as the presence of Ca-loaded polyphosphates possibly could harm the functioning of the arbuscules.

The elevated concentrations of Fe and Mn that we found in the mycorrhizal plants resulted mainly from very high concentrations in the root fraction (data not shown). They are in line with Bi et al. (2003) and Nogueira et al. (2007). Improved plant root development and acquisition of P may be involved in enhanced Fe acquisition by mycorrhizal plants (Clark and Zeto 1996).

Strong mycorrhizal effects on chickpea were also observed when looking at the nutrient uptake. We found an increase of P, K, Mg, Fe, Mn, Zn, and Cu uptake after AMF inoculation partly as a reflection of differences in dry matter. Only changes in Ca and N uptakes with AMF chickpea were relatively

Table 5. P concentrations and Mn concentrations and uptake in total chickpea biomass as affected by treatments without (M–) or with (M+) AMF inoculation in interaction with soil sterilization (S+ sterilized soil or S– non-sterilized soil) in 2007 (n = 10)

		Nutrient element		
		P	Mn	
		(g/kg)	(mg/kg)	(µg/pot)
M+	S+	1.45	166.62	1879.8
	S–	1.46	115.00	1076.6
M–	S+	0.98	120.31	917.1
	S–	1.35	97.44	694.6
$LSD_{0.05}$		0.17	17.46	324.3

small. Similar positive effects of AMF on nutrient uptake in legumes were reported earlier (Zaidi et al. 2003, Ilbas and Sahin 2005, Lin et al. 2007). The higher P, K, Mg, Fe, Mn, Zn and Cu uptake of the mycorrhizal chickpea plants in parallel with the lack of difference in N uptake of mycorrhizal and non-mycorrhizal plants in a growth substrate with adequate N levels might indicate a positive effect of AMF on dry matter by increasing the uptake of nutrients other than N (Jia et al. 2004). Our results are in accordance with those of previous work on chickpea by Akhtar and Siddiqui (2007) showing no effect on N uptake but increased P and K uptake in mycorrhizal plants.

Importance of soil sterilization. Competition between inoculated AMF, indigenous mycorrhiza and other soil microorganism in non-sterilized soil may affect the AMF impact (Baas 1990). But we found only little interactions of AMF inoculation with soil sterilization on nutrient acquisition of chickpea in 2007 despite substantial differences in root colonization. Only with Mn the artificially introduced strains of Symbivit® enhanced concentration and uptake in sterilized soil more pronounced than in combination with the indigenous population. Concentrations of P, on the other hand, were elevated above the level of non-colonized plants (M– S+ treatment) by inoculated and indigenous strains to a similar degree, although Symbivit® increased colonization substantially in both soils by +25–26% of roots colonized. Crops sometimes benefit from indigenous or introduced symbionts with little correlation to the degree of root colonization (Al-Raddad 1991). Contrasting to a study with alfalfa by Biró et al. (2000), we did not find that inoculation with an AMF strain was more supportive on N, P and K uptake if indigenous AMF were excluded.

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