

# The effects of arbuscular mycorrhiza and nitrogen nutrition on growth of chickpea and barley

Einflüsse von arbuskulärer Mykorrhiza und Stickstoff-Ernährung auf das Wachstum von Kichererbsen und Gerste

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## Summary

There is increasing evidence for the promoting effects of arbuscular mycorrhiza fungi (AMF) on the growth of practically important crops. The present study evaluates the effects of inoculating AMF on growth of chickpea and barley based on a series of pot experiments during 2 years. A range of soil biological and chemical conditions was used to test the AMF treatment in interaction with indigenous microbes (sterilized vs. non-sterilized soil), application of additional fertilizer N or co-inoculation of chickpea with nitrogen-fixing rhizobia. The effect of treatments on colonization by AMF, rhizobial nodule number and weight, plant dry matter and soil mineral N were determined in randomized complete block designs with five replications using a chernozem topsoil of silty loam in a 1:1 mixture with sand as basic substrate. Inoculated plants were effectively colonized by AMF and attained more dry matter than control plants in both sterilized and non-sterilized soil, but colonization levels varied substantially between years. Both, chickpea and barley showed similar growth enhancement, though the AMF colonization level was lower with barley than with chickpea. The non-sterilized soil contained no natural rhizobia strains suitable for chickpea infection, but with rhizobia inoculation nodules developed. However, no growth effect was obtained. The level of soil mineral N did not affect AMF performance. Presumably nitrogen was no growth limiting factor in our experiments. Our study confirms the growth enhancing potential of AMF inoculation on both chickpea and barley.

**Key words:** arbuscular mycorrhiza fungi (AMF), chickpea, barley, nitrogen nutrition, growth

## Zusammenfassung

Wachstumsfördernde Wirkungen arbuskulärer Mykorrhiza (AM) von praktischer Relevanz werden zunehmend anerkannt. Die vorliegende Studie untersucht die Effekte einer Inokulation mit AM auf Kichererbsen und Sommergerste anhand einer Serie von Gefäßversuchen über 2 Jahre. Unter verschiedenen bodenbiologischen und bodenchemischen Bedingungen wurde die AM-Inokulation in Wechselwirkung mit der natürlichen Boden-Mikroflora (sterilisierten bzw. nicht-sterilisierten Boden), mit mineralischer N-Düngung oder mit der Co-Inokulation von N-fi-

xierenden Rhizobien geprüft. Die Auswirkungen der Versuchsglieder auf AM-Wurzelkolonisierung, Knöllchenzahl und -gewicht, Pflanzen-Trockenmasse sowie Mineralstickstoffgehalt des Bodens wurden in vollständig randomisierten Blockanlagen bei fünffacher Wiederholung erhoben. Das Substrat war ein Tschernosem-Oberboden aus schluffigem Lehm in einer 1:1 Mischung mit Quarzsand. Die inokulierten Pflanzen wurden effektiv von AM besiedelt und produzierten mehr Trockenmasse als die Kontroll-Pflanzen sowohl in sterilisiertem als auch in nicht-sterilisiertem Boden, aber die Kolonierungsgrade variierten jahresabhängig. Kichererbse und Gerste zeigten eine ähnliche Wachstumsförderung, obwohl die Kolonisierung der Gerste geringer ausfiel als bei Kichererbsen. Der nicht-sterilisierte Boden enthielt keine natürlichen Rhizobien, die Kichererbsen infizieren konnten, aber nach Inokulation wurden Wurzelknöllchen beobachtet, jedoch traten keine Wachstumseffekte auf. Der Mineralstickstoffgehalt des Bodens beeinflusste die AM-Wirkung nicht. N war anscheinend kein begrenzender Wachstumsfaktor in den Versuchen. Unsere Ergebnisse bestätigen die mögliche Wachstumsförderung durch AM bei Kichererbsen und Gerste.

**Schlüsselworte:** arbuskuläre Mykorrhiza, Kichererbse, Gerste, Stickstoffernährung, Wachstum

## Introduction

Arbuscular mycorrhizal fungi (AMF) are present in most agro-ecosystems. These obligate mutualistic symbionts colonize the roots of the vast majority of plant species, including most crops (SHARIF & MOAWAD 2006). The extraradical mycorrhizal hyphal network provides a larger absorptive surface than root hairs alone and thus improves the uptake of relatively immobile ions from soil such as phosphate, copper and zinc. In addition, AMF colonized plants are known to be more tolerant to several biotic and abiotic stresses such as toxic metals, root pathogens, drought, high soil temperature, saline soils, adverse soil pH and transplanting than non-AMF colonized plants (PARASKEVOPOULOU PAROUSSI et al. 1997, SMITH & READ 1997, RUIZ-LOZANO et al. 2001, RABIE & ALMADINI 2005, TURKMEN et al. 2008). Furthermore, AMF may play a role in the formation of stable soil aggregates, building up a macro pore structure of soil that allows for easier penetration of water and air and prevents erosion (PIOTROWSKI et al. 2004). Ad-

ditional inoculation with selected AMF strains, which are available as commercial products, often yields better growth promotion than indigenous AMF populations (DA SILVEIRA & LIMA 1996, SALAMI et al. 2005).

Chickpea (*Cicer arietinum* L.) holds a unique position among pulse crops due to its seed protein content and wide adaptability in ecologically diverse environments. It plays a significant role in farming systems as a substitute for fallow in cereal rotations, where it contributes to the sustainability of production and reduces the need for N fertilization through fixing atmospheric nitrogen and breaking gramineous crop disease cycles (JODHA & SUBBA RAO 1987). Spring sown barley (*Hordeum vulgare* L.) is a typical non-legume alternative to chickpea in grain crop rotations due to similar vegetation periods. It is often used as a reference crop for estimating N fixation of grain legumes (DOUGHTON et al. 1995, CARRANCA et al. 1999). Recently, CHAURASIA & KHARE (2005) showed that an intensive AMF root colonization of barley fosters plant growth and dry matter production.

The positive effects of the inoculation of AMF on plant growth are generally attributed to the improved uptake of nutrients of low mobility, especially phosphorus (SUBBA RAO et al. 1986, SINGH & TILAK 1989, WEBER et al. 1993, MARSCHNER & DELL 1994). However, some evidence of the capability of AMF hyphae to take up and transport  $^{15}\text{NO}_3$  to the host plant has also been reported (BAGO et al. 1996). JOHANSEN et al. (1996) demonstrated that external hyphae of an AM fungus were able to take up  $\text{NH}_4$  and  $\text{NO}_3$  and to assimilate these molecules into the pool of free amino acids. This is supported by several findings that AMF hyphae can absorb, translocate and utilize  $\text{NH}_4$  (AMES et al. 1983, SMITH et al. 1985, KESSEL et al. 1985, CLIQUET & STEWART 1993, JIN et al. 2005). The two most important sources of inorganic nitrogen for plants, and potentially for AMF, are nitrate ( $\text{NO}_3$ ) and ammonium ( $\text{NH}_4$ ) ions. In agricultural soils  $\text{NO}_3$  usually predominates because of the rapid nitrification of  $\text{NH}_4$ .  $\text{NO}_3$  is not adsorbed by soil colloids and is mobile in the soil solution, thus mass flow to roots allows uptake to be maintained at rates dependent on the root absorbing power (SMITH & READ 1997). In contrast,  $\text{NH}_4$  belongs to the non-mobile nutrient compounds. Additional N uptake in nodulated legumes due to AMF colonization has been observed (BAREA et al. 1987, BIRÓ et al. 2000). This can result from increased rhizobial  $\text{N}_2$  fixation (as expected because of the better phosphorus supply by AMF) or increased N uptake from soil by the AMF hyphal network (SUBBA RAO et al. 1986). There are scarce data on the effects of N fertilization on chickpea in absence of rhizobia. However, a few existing studies show inconsistent results regarding growth, yield and  $\text{N}_2$  fixation responses of chickpea to N fertilization in natural production environments and rotation systems (e. g. EL-GHANDOUR & GALAL 2002, WALLEY et al. 2005, GAN et al. 2008).

In addition to AMF, soils also contain various antagonistic and beneficial bacteria such as root pathogens, plant growth promoting rhizobacteria, including free-living and symbiotic N-fixers, and mycorrhiza helping bacteria (KHAN 2006). It has been found that inoculation with AMF (*G. fasciculatum*) was effective in increasing N, P and K content of alfalfa shoots as long as indigenous AMF were excluded (BIRÓ et al. 2000). The contrast between a marked response of the legume *Sesbania grandiflora* to inoculation of sterilized soil with *Glomus fasciculatum* versus a more modest response to inoculation of non-sterilized soil with the fungus suggests that in non-sterilized soils, inoculated AMF interact not only with indigenous AMF but also with other microorganisms (HABTE & AZIZ 1985). Compatible combinations of inoculated microbes, such as associative

(*Azospirillum brasilense*) and/or symbiotically (*Rhizobium meliloti*) nitrogen-fixing bacteria plus AMF (*Glomus fasciculatum*), resulted in an enhanced effect on plant growth, though *Azospirillum* in the absence of *Rhizobium* was antagonistic to AMF (TSIMILLI-MICHAEL et al. 2000). ZAIDI et al. (2003) showed that plant yield and nutrient uptake were further augmented by the addition of the AM fungus *Glomus fasciculatum* combined with *Rhizobium* sp. and phosphate solubilising microorganisms (PSM) of *Pseudomonas striata*. In contrast a negative effect occurred on all the considered parameters when *G. fasciculatum* was added to the combination of *Rhizobium* sp. and another PSM, *Penicillium variable*. The effects of different co-inoculations can be much more pronounced in sterilized soil compared to non-sterilized controls. This reveals a buffering capacity of the control soils arising from the presence of indigenous microflora (BIRÓ et al. 2000). Consequently, the growth promoting effect of AMF inoculation depends also on the effectiveness and infectivity of indigenous microbes and on the interactions between the main community members, i. e. between indigenous microbes, inoculated AMF and host species. Soil sterilization can be used to exclude indigenous microbes.

The aims of the present study were (i) to evaluate the effects of inoculation of AMF on growth of chickpea in absence or presence of rhizobia due to soil sterilization and subsequent inoculation, (ii) to identify the interactions of indigenous soil microorganisms with AMF and rhizobia without soil sterilization and (iii) to compare the effects of AMF on chickpea and barley with and without soil sterilization. In all experiments, AMF inoculation was tested in combination with or without application of additional mineral fertilizer N.

## Material and Methods

Experiments in Mitscherlich pots (6.3 l soil volume) were carried out during spring and summer 2006 and 2007 under sheltered conditions in a glasshouse in the city of Vienna, Austria, with the possibility to transfer the pots into ambient conditions of a fence house under favorable weather conditions (Tab. 1). The experiments were in a randomized complete block design with five replications. The factorial design included the following factors:

- Year (2006 or 2007)
- Crop species (chickpea or barley)
- AMF inoculation (M+ or M-, i.e. with or without inoculum "Symbivit®")
- Nitrogen nutrition (N- R-, N+ R- or N- R+, i.e. only soil supply, with mineral fertilizer at 314 mg N per pot (equivalent to 100 kg N ha<sup>-1</sup>) or with inoculation of rhizobia, the latter not for barley)
- Soil sterilization (with or without sterilization of soil, the latter only in 2007)
- Harvest date (H1 or H2, i.e. at flowering or at physiological maturity).

Combinations of the factors resulted in four orthogonal subsets of data, subsequently indicated as experimental units I, II, IIIa and IIIb that were separately submitted to analyses of variance.

Chickpea (commercial seeds of unidentified Kabuli genotype) and barley (cv. Xanadu) seeds were sown in pots filled with a mixture (1:1) of sterilized soil and sand. The soil was a chernozem topsoil of silty loam taken from the experimental farm Gross-Enzersdorf of BOKU University. The soil-sand substrate was analyzed (cf. Tab. 1) and subsequently sterilized (105°C, 24 h) before sowing. Additionally, in 2007 we studied the effects of soil sterilization

Tab. 1: Characteristics of pot experiments  
*Rahmenbedingungen der Gefäßversuche*

Growing season	2006		2007
pH value (CaCl <sub>2</sub> )	7.5		7.5
NO <sub>3</sub> -N	5.6 mg kg <sup>-1</sup> (25 kg N ha <sup>-1</sup> )		10.7 mg kg <sup>-1</sup> (48 kg N ha <sup>-1</sup> )
Plant available P (CAL)	106 mg kg <sup>-1</sup>		138 mg kg <sup>-1</sup>
Plant available K (CAL)	191 mg kg <sup>-1</sup>		248 mg kg <sup>-1</sup>
Plant available Mg (CaCl <sub>2</sub> )	94 mg kg <sup>-1</sup>		115 mg kg <sup>-1</sup>
Crop	chickpea, barley	chickpea, barley (only with non-sterilized soil)	
Date of sowing	25 and 26 April		23 and 24 April
Harvest date at flowering	28 June		20 June
Harvest date at maturity	25-27 July	20 July (barley) and 7 August (chickpea)	

(with or without sterilization). In all treatments, chickpea or barley seedlings were manually thinned out to three or ten seedlings per pot, respectively, one week after emergence.

Inoculation of the AMF treatments was done by adding the AMF product "Symbivit®" (Symbio-m, s.r.o., Lanškroun, CZ) to the pots at planting. It was placed below seeds at a rate of approximately 5 g for a group of seeds which were later thinned to one seedling. The inoculum consisted of an inert carrier (a mix of slate, zeolite and clay) that contained reproductive particles (spores, mycelium and colonised root fragments) from six different strains of *Glomus* spp. (*G. intraradices* BEG 98, *G. mosseae* BEG 99, *G. claroideum* BEG 93, *G. microagregatum* BEG 56, *G. caledonium* BEG 97, *G. etunicatum* BEG 92; BEG = La Banque Européenne des Glomales, International Institute of Biotechnology, Kent, GB; <http://www.kent.ac.uk/bio/beg>) isolated from a range of soils from various ecosystems.

Pots with N application (N+) received an amount of 314 mg N per pot, equivalent to 100 kg N ha<sup>-1</sup>, as a calcium ammonium nitrate (27% N) solution one week after emergence. For inoculation with rhizobia (R+) we used the water suspension "Radicin®" (Jost GmbH, Iserlohn, D) one week after emergence. Tap water (4 mg NO<sub>3</sub> l<sup>-1</sup>) was supplied during the vegetation period daily if necessary to avoid any drought stress.

Plants were harvested at flowering stage (H1) or at physiological maturity (H2) by removing them completely from the pots. Subsequently plants were divided into shoots and roots, at maturity shoots were further divided into straw and pods or ears, respectively. Root samples were washed out carefully with water and a sieve (250 µm mesh size). For the later evaluation of mycorrhizal colonization the roots were stored in an alcoholic solution (50% ethanol). Shoot and root samples were dried at 105°C for 24 h and amounts of dry matter were measured gravimetrically.

Before dry matter determination of roots, root segments (1 cm in length) below the upper 2 cm of the roots had been sampled to estimate root colonization by AMF. The roots were cleared with a 10% KOH solution and stained with a 5% ink solution (Shaeffer jet black + acetic acid). Evaluation of AMF colonization was done under a microscope by a grid line method according to VIERHEILIG et al. (1998).

Soil samples were taken during harvesting by mixing the soil of each pot after the plants had been removed and putting a representative sample into a plastic bag. The bags were immediately frozen at -20°C. Subsequently the content of soil mineral N (nitrate and ammonium N) was extracted by a CaCl<sub>2</sub> solution and measured by a photometer

method on a FIASTAR 5000 apparatus (FOSS GmbH, D). In all soil samples NH<sub>4</sub> concentrations were very low, i. e. below 0.1 kg NH<sub>4</sub>-N ha<sup>-1</sup>, thus we report only NO<sub>3</sub>-N results.

The statistical analysis of all observations was done by the procedure MIXED of the SAS software. For significant factorial effects, the t-test was used to compare means and least significant differences (LSD) were calculated. The significance threshold was assumed at p=0.05.

## Results

The results section starts with a table showing characteristics of soil biological and chemical conditions of the experiments as obtained by inoculation, fertilization and soil sterilization treatments (Tab. 2). The subsequent presentation of dry matter results is based on the analyses of variance that have been performed for four experimental units, i.e. orthogonal subsets of data. Due to our research objectives, significant interactions of AMF inoculation and nitrogen nutrition with soil sterilization or crop species were of primary interest. These have been frequently modified by environmental conditions or plant development, i.e. by year or harvest date. Due to the heterogeneity of significant interaction levels, and because interactions between AMF inoculation and nitrogen nutrition were only rare, the general results are presented in two comprehensive figures (Fig. 1, 2), giving an overview about AMF or nitrogen nutrition effects, respectively. These are supplemented by individual specific effects of relevance for our objectives.

### Soil biological and chemical conditions of the experiments

The inoculation with AMF has been successful, because all inoculated plant samples were substantially colonized (Tab. 2). The colonization level was higher in 2006 than in 2007, without than with soil sterilization and with chickpea compared to barley. Without sterilization, the soil obviously contained indigenous populations which were able to colonize chickpea and barley. Yet, additional inoculation increased colonization also in non-sterilized soil. After soil sterilization without AMF inoculation no colonized roots were found at all. Nitrogen fertilization had no effect on mycorrhizal root colonization (data not shown).

There was a significant interaction between harvest date and year on percentage of AMF colonization for chickpea in sterilized soil. The colonization levels varied in different years from flowering to maturity. In 2006 chickpea roots

Tab. 2: Characterization of soil biological and chemical conditions, i. e. percentage of AMF colonized roots, rhizobial nodule dry weight and number (all on average across two harvest dates) and soil NO<sub>3</sub>-N at maturity. Means and standard deviations (in brackets) indicated according to the subsets of data submitted to statistical analysis (experimental units I, II, IIIa, IIIb). Treatments without (M-) or with (M+) AMF inoculation, without (N-) or with (N+) mineral fertilizer, without (R-) or with (R+) rhizobia inoculation

Charakterisierung der bodenbiologischen und chemischen Bedingungen der Versuche, d. h. prozentuale Wurzelbesiedelung mit AM, Rhizobien-Knöllchengewicht und -zahl (jeweils im Mittelwerte über beide Erntetermine) sowie NO<sub>3</sub>-N im Boden zur Vollreife. Mittelwerte und Standardabweichungen (in Klammern) getrennt nach den orthogonalen Datensätzen für die Varianzanalysen (I, II, IIIa, IIIb). Versuchsglieder ohne (M-) oder mit (M+) AM Inokulation, ohne (N-) oder mit (N+) Mineraldüngung, ohne (R-) oder mit (R+) Rhizobien Inokulation

Unit	Year	Species	Soil sterilization	AMF Colonization (%)		Nodule weight (mg per pot)			Soil NO <sub>3</sub> -N (mg kg <sup>-1</sup> )			
				M+	M-	N- R-	N+ R-	N- R+	N- R-	N+ R-	N- R+	
I	2006	Chickpea	with sterilization	54.5 (11.8)	0 (0)	0 (0)	0 (0)	46.1 (30.1)	41 (22)	12.0 (14.0)	25.3 (19.2)	6.1 (3.6)
	2007			24.9 (19.8)	0 (0)	0 (0)	0 (0)	3.2 (8.2)	5 (4)	15.1 (6.5)	31.6 (5.9)	11.5 (6.0)
II	2007	Chickpea	with sterilization	24.9 (19.8)	0 (0)	0 (0)	0 (0)	3.2 (8.2)	5 (4)	15.1 (6.5)	31.6 (5.9)	11.5 (6.0)
			without sterilization	44.1 (22.4)	17.8 (12.3)	0 (0)	0 (0)	52.5 (96.8)	18 (18)	5.1 (4.7)	14.6 (4.8)	3.5 (3.5)
IIIa	2006	Chickpea	with sterilization	55.1 (12.4)	0 (0)					12.0 (14.0)	25.3 (19.2)	
			Barley	17.7 (10.0)	0 (0)					2.9 (2.1)	3.0 (1.7)	
IIIb	2007	Chickpea	without sterilization	43.5 (20.8)	19.7 (13.3)			for chickpea: see above for barley: not applicable		5.1 (4.7)	14.6 (4.8)	not tested
			Barley	17.6 (9.0)	6.6 (6.9)					6.7 (1.7)	14.1 (7.3)	

showed the highest colonization rate at the flowering stage (58%) that decreased to 51% at maturity, while in 2007 the higher mycorrhizal colonization rate of 35% was observed at maturity time, compared to only 14% at flowering. Also with barley in 2006 or 2007, colonization increased from 14% or 6% at flowering to 21% or 18% at maturity, respectively.

Similar to AMF, rhizobia inoculation resulted in nodule production while without rhizobia addition no nodules were observed. Missing nodulation also in non-sterilized soil indicates that the soil from our experimental station was not colonized by rhizobia strains capable of chickpea infection. The inoculation success was much more pronounced in 2006 and also in non-sterilized soil.

Soil NO<sub>3</sub>-N concentrations at final harvest were slightly higher in 2007 than in 2006, but the difference is about the same as the difference in initial concentrations of the substrates (cf. Tab. 1). Nitrate concentrations were substantially increased after addition of fertilizer N. They were generally lower under barley or in non-sterilized soil compared to chickpea or sterilized soil, respectively. Under chickpea we observed a tendency of lower soil NO<sub>3</sub>-N concentrations after rhizobia inoculation compared to the untreated control, i. e. N- R- (data not shown).

### Mycorrhizal effects

Analyzing chickpea in sterilized soil in two years (exp. unit I), the interaction of mycorrhizal inoculation x harvest date x year showed that mycorrhiza increased total dry matter in both years, but this enhancing effect was significant only on the first harvest in 2006 and on the second harvest in

2007 (Fig. 1). This resulted from significant differences in all plant fractions (i. e. roots, shoots and pods) between inoculated and non-inoculated plants.

Comparing chickpea 2007 in sterilized vs. non sterilized soil (unit II), the positive mycorrhizal effect at maturity can be confirmed also for the non-sterilized soil. Only pod yield was not improved.

With regard to the comparison of barley and chickpea, in 2006 in sterilized soil (unit IIIa) AMF inoculation did not affect growth. Contrastingly, in 2007 in non-sterilized soil (unit IIIb) total dry matter and all plant fractions were enhanced by AMF with slightly stronger effects on barley than on chickpea. For pod/ear dry matter, no significant differences in the pod dry matter were observed between AMF inoculated and non-inoculated chickpea, while inoculated barley showed higher ear dry matter than non-inoculated plants. There was a favorable effect of AMF inoculation on barley shoot/straw dry matter at both sampling dates, while chickpea straw production was only enhanced at maturity.

AMF also affected soil NO<sub>3</sub>-N concentrations under chickpea, but only in 2007 in sterilized soil (Tab. 3). Only under these conditions we found substantially more NO<sub>3</sub>-N after AMF inoculation than in non-inoculated pots.

### N nutrition effects

With chickpea in sterilized soil (unit I), we found hardly any consistent effect of nitrogen nutrition on chickpea growth, neither due to fertilizer N nor to rhizobial infection (Fig. 2). Only root dry matter was significantly affected by nitrogen nutrition. On average across years and harvest dates, mineral N fertilizer application (R-N+) reduced root

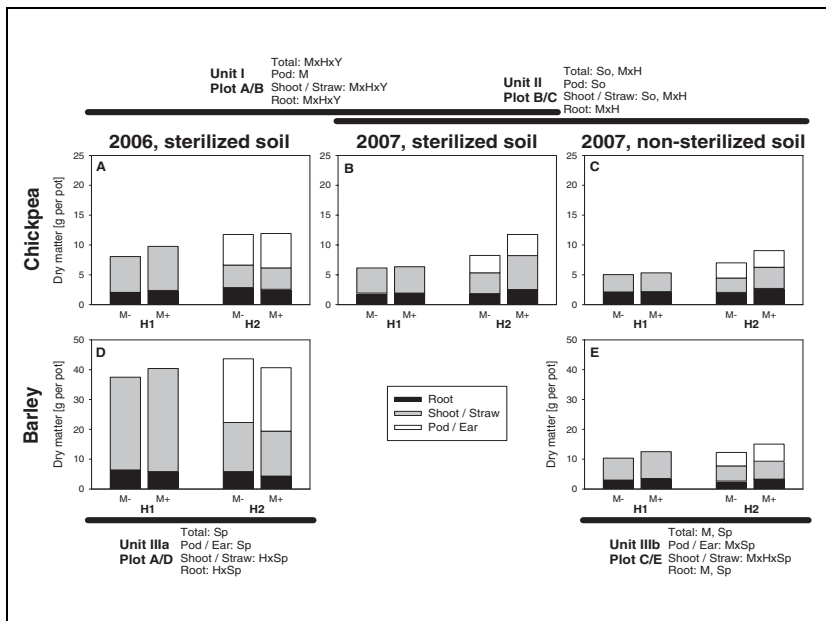


Fig. 1: Dry matter of crops, divided into fractions, as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with year (Y), soil sterilization (So), crop species (Sp) and harvest date (H1 flowering, H2 physiological maturity) in four experimental units (as indicated with black bars). For each unit the significant effects included in the figures are indicated. Note that axis scales for chickpea and barley are different.

*Trockenmasse der Pflanzen, aufgeteilt in Fraktionen, in Abhängigkeit von AM-Inokulation (M-/M+) in Wechselwirkung mit Jahr (Y), Bodensterilisation (So), Pflanzenart (Sp) und Erntetermin (H1 Blühbeginn, H2 physiologische Reife) in vier Datensätzen (s. Beschriftung und schwarze Balken). Für jeden Datensatz sind die in der Abbildung enthaltenen signifikanten Effekte angegeben. Die unterschiedliche Achsenskalierung für Kichererbse bzw. Gerste ist zu beachten.*

growth compared to the unfertilized crops with or without rhizobia inoculation.

Comparing the soils in 2007 (unit II), shoot/straw and pod yields were generally higher in sterilized soil. Chickpea showed different reactions to N nutrition treatments in total and root dry matter. The root depressing effect of mineral fertilization did not appear in the non-sterilized soil and total dry matter was even enhanced by the fertilizer. On the other hand in sterilized soil rhizobia inoculation caused the highest root and total dry matter.

With view to the two species (units IIIa, b), barley growth was strongly enhanced by mineral fertilizer in contrast to chickpea in both soil treatments and years, respectively.

**Interaction effects between AMF inoculation and nitrogen nutrition**

Chickpea pod yield across two years (unit I) in the presence of AMF colonization was improved by fertilizer N while pod yield of rhizobia treated plants was lower (Fig. 3). Contrastingly, without AMF inoculation rhizobia infected plants produced more pod dry matter than those with fertilizer N.

On average across both species, chickpea and barley, in 2006 in sterilized soil (unit IIIa), root growth of AMF colonized plants was hardly affected by N fertilization, while without AMF it was significantly enhanced by fertilizer N (Tab. 4).

**Discussion**

**Success of inoculations**

In all experiments we were able to establish a suitable level of AMF colonization, although the percentage of AMF colonized roots was markedly affected by environment and crop species. Seasonal patterns in the formation of mycorrhiza have also been found to vary considerably from year to year (SANDERS & FITTER 1992, MUTHUKUMAR & UDAIYAN 2002). On the other hand, non-inoculated pots with sterilized soil were obviously kept completely free of AMF and also in non-sterilized soil, a distinct increase in AMF colonization was observed after inoculation.

As chickpea had never been grown before on the experimental fields we had used as soil source for our pot experiments, it was not surprising that the non-sterilized soil contained no suitable rhizobia strains for chickpea infection. The inoculation with rhizobia ("Radicin®"), however, induced nodulation with substantially better results in 2006 but also in 2007.

**Mycorrhiza and N nutrition effects on chickpea**

AMF colonization caused substantial growth improvement by up to +43% total dry matter at maturity as compared to the non-inoculated control. Positive effects of AMF inoculation on chickpea and other legume species have been explained by an enhancement of root hair length and mycorrhizal mycelium length at early stages of plant develop-

Tab. 3: Soil NO<sub>3</sub>-N concentrations under chickpea as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with year or soil sterilization. Experimental units I and II, means across harvest dates  
*NO<sub>3</sub>-N im Boden unter Kichererbse in Abhängigkeit von AM-Inokulation (M-/M+) und Jahr bzw. Bodensterilisation. Datensätze I und II, jeweils im Mittel über beide Erntetermine*

Exp. unit	Year	Soil sterilization	Soil NO <sub>3</sub> -N (mg kg <sup>-1</sup> )		LSD
			M+	M-	
I	2006	with sterilization	10.8	14.8	4.0
	2007		22.2	14.2	
II	2007	without sterilization	9.1	9.3	2.6

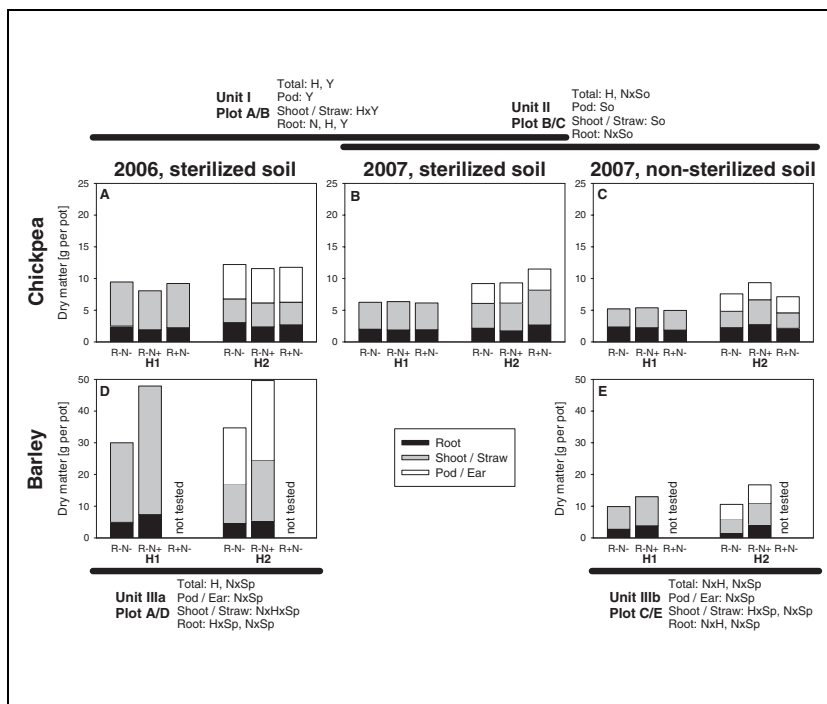


Fig. 2: Dry matter of crops, divided into fractions, as affected by nitrogen nutrition (N), i.e. control (N- R-), with mineral fertilizer (N+ R-) or with rhizobia inoculation (N- R+), in interaction with year (Y), soil sterilization (So), crop species (Sp) and harvest date (H1 flowering, H2 physiological maturity) in four experimental units (as indicated with black bars). For each unit the significant effects included in the figures are indicated. Note that axis scales for chickpea and barley are different. Trockenmasse der Pflanzen, aufgeteilt in Fraktionen, in Abhängigkeit von der Stickstoff-Ernährung (N), d. h. Kontrolle (N- R-), mit Mineraldüngung (N+ R-) oder mit Rhizobien-Inokulation (N- R+), in Wechselwirkung mit Jahr (Y), Bodensterilisation (So), Pflanzenart (Sp) und Erntetermin (H1 Blühbeginn, H2 physiologische Reife) in vier Datensätzen (s. Beschriftung und schwarze Balken). Für jeden Datensatz sind die in der Abbildung enthaltenen signifikanten Effekte angegeben. Die unterschiedliche Achsenskalierung für Kichererbse bzw. Gerste ist zu beachten.

ment (SCHWEIGER et al. 1995, WEBER et al. 1993). The present results confirm a positive effect on root growth, although the growth promotion on shoots in general was more pronounced.

AMF are known to be effective in increasing nutrient uptake, particularly phosphorus, and biomass accumulation of many crops in soils low in phosphorus (TURK et al. 2006) or soils that fix phosphorus due to a high concentration of calcium and high pH values. In those situations, organic acids produced by AMF may partially explain enhanced nutrient uptake by the roots of mycorrhizal plants (SHARIF & MOAWAD 2006). The soil material in the present study was not low in plant available phosphorus, however of an elevated pH (cf. Tab. 1).

AMF inoculation increased the soil NO<sub>3</sub>-N content at maturity in sterilized soil in 2007 under chickpea. This might be explained because AMF plants have access to N pools in the soil which are not equally available to non-mycorrhizal plants (AMES et al. 1984), thus saving plant available soil N.

In contrast with the AMF treatment, we found no significant growth enhancement due to rhizobial infection. As also mineral N fertilization of chickpea did not improve dry matter production, N was obviously sufficiently available in control pots and not growth limiting.

After inoculation, nodulation of chickpea varied with soil sterilization and between years. Nodules were not always observed. When they were present their number of-

ten exceeded 10 per plant, but most of them were of small size and apparently ineffective. This suggests that compatible, effective rhizobia were not present in the inoculum product or their environmental demands were not fulfilled. Bradyrhizobium strains that infect chickpea are specific and rarely exist especially in soil where chickpea were not grown before (EL HADI & ELSHEIKH 1999, DATE 2000). AOUANI et al. (2001) showed that some rhizobial species were able to nodulate chickpea, but the symbiosis they formed took a longer time to establish than when formed with specific mesorhizobia, and was ineffective, which indicated that they were not really compatible. Ineffective or hardly effective cross nodulation has been reported for other legumes, too (MUTCH & YOUNG 2004, NANDASENA et al. 2004). It is evident that there was hardly any cross-inoculation between nodulating rhizobia of *C. arietinum* and those infecting other legumes. Only strains of *Sesbania-Rhizobium* were found to be able to form nodules on *C. arietinum* (GAUR & SEN 1979).

Although some minor interaction effects between AMF and N nutrition were significant, they were neither important nor consistent across experimental units and thus cannot be generalized.

**Effects of soil sterilization**

The additional colonization potential of the natural AMF population present in non-sterilized soil resulted in higher

Tab. 4: Dry matter (D.M.) of roots as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with nitrogen nutrition without (R-N-) or with (R-N+) mineral fertilizer. Experimental units IIIa, means across species and harvest dates Wurzeltrockenmasse in Abhängigkeit von AM-Inokulation (M-/M+) und Stickstoff-Versorgung ohne (R-N-) oder mit (R-N+) Mineraldüngung

Unit	Year	Soil sterilization	Nitrogen nutrition	Root D.M. (g per pot)		LSD
				M+	M-	
IIIa	2006	with sterilization	R-N-	3.97	3.58	0.90
			R-N+	3.67	4.90	

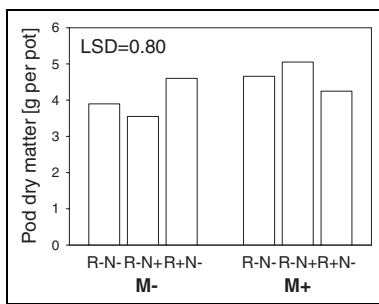


Fig. 3: Dry matter of chickpea pods as affected by treatments without (M-) or with (M+) AMF inoculation in interaction with nitrogen nutrition (N), i.e. control (N- R-), with mineral fertilizer (N+ R-) or with rhizobia inoculation (N- R+). Experimental unit I, sterilized soil, harvest date H2, means across years

Trockenmasse der Kichererbsen-Hülsen in Abhängigkeit von AM-Inokulation (M-/M+) und Stickstoff-Ernährung (N), d. h. Kontrolle (N- R-), mit Mineraldüngung (N+ R-) oder mit Rhizobien-Inokulation (N- R+). Datensatz I, sterilisierter Boden, Erntetermin H2, Mittelwerte über die Jahre

colonization rates, but did not additionally improve dry matter production. This result indicates that native AMF was less effective to promote plant growth than the commercial product "Symbivit®". On the contrary, other authors had observed substantial competition of the indigenous microflora in non-sterilized soil, which greatly reduced the efficacy of applied mycorrhizal inoculum (ABBOTT & ROBSON 1981, IBLBJEN et al. 1996, BIRÓ et al. 2000). This is not confirmed by our results.

#### Comparing chickpea with barley

Barley produced much more biomass per pot than chickpea, which was presumably biased by the different densities of ten barley plants as compared to three chickpea plants per pot. The density chosen for chickpea had been based on a much stronger growth of individual plants in previous field experiments (WICHMANN et al. 2007). Improved plant growth of chickpea and barley was observed following inoculation with AMF. These positive effects were accompanied by variable percentages of root colonization in years or soil and crop types.

Our findings of relatively high levels of AMF root colonization in chickpea compared with barley differ from a study on the same plant species by SHARIF & MOAWAD (2006). Although colonization levels of barley were generally lower, the fostering of growth was even slightly more pronounced than with chickpea. CHAURASIA & KHARE (2005) also found more growth promotion with barley as compared to legume species but in their study also barley root colonization was higher, while ZHU et al. (2000) reported less colonization of grass compared with clover roots.

It seems that barley was more efficiently supported by mycorrhiza than chickpea because after inoculation with AMF it produced finally additional ear dry matter, while no additional enhancement of chickpea pod yield was observed. This finding is in agreement with WEBER et al. (1993) who reported that 'high mycorrhizal' chickpea plants at maturity had a lower harvest index compared to 'low mycorrhizal' plants and tended to give less seed yield despite greater shoot biomass. They hypothesized that the colonization with AMF improved P uptake and growth of chickpea early in the season thereby increasing water stress during seed development. But water shortage must not be suspected in our pot trials.

#### Conclusions

The percentage of AMF colonized roots was markedly affected by environment, crop species and sterilization of soil. However, AMF inoculation of chickpea and barley consistently led to AMF root colonization and subsequently enhanced dry matter production. This growth stimulation was hardly related to nitrogen nutrition, since nitrogen was presumably not a growth limiting factor even in control pots (N- R-) of our experiments. Native AMF in non-sterilized soil were less effective to promote plant growth than the commercial product "Symbivit®" and did not reduce the efficacy of applied mycorrhizal inoculum.

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