

Response of winter wheat to arbuscular mycorrhizal fungal inoculation under farm conditions

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Abstract: The effect of arbuscular mycorrhizal (AM) fungi inoculation was investigated on two winter wheat cultivars (*Triticum aestivum* var. Mv Nádor and var. Genius) grown under farm conditions in the neighbourhood of Nagyhörcsök in 2016. The soil was a chernozem with lime deposits (WBR classification: Calcic chernozem) with a mean humus content of 2.73%, AL-soluble P₂O₅ and K₂O concentrations of 181 mg kg⁻¹ and 149 mg kg⁻¹ and a pH_(KCl) value of 7.27. The AM inoculum contained reproductive units of *Rhizophagus irregularis* (previously *Glomus intraradices*) and *Glomus mosseae* (syn. *Funneliformis mosseae*). In addition to soil inoculation, some of the treatments were also given mineral fertiliser treatment (130 kg N ha⁻¹, 78 kg P₂O₅ ha⁻¹, 60 kg K₂O ha⁻¹).

Both AM inoculation and mineral fertiliser treatment were found to have a significant effect on the yield (at the p<0.05 level). The yield of plots with mycorrhizal inoculation averaged 8.17 t ha⁻¹, which was higher than that of non-inoculated plots (7.52 t ha⁻¹), while the yield of plots with fertiliser treatment averaged 8.31 t ha⁻¹, as compared with 7.38 t ha⁻¹ for non-fertilised plots. The yield-enhancing effect of AM inoculation was only manifested in plots given no mineral fertiliser. Plant protection measures were the same in all the treatments.

The results and the conclusions drawn from them were based on the data of a single year (2016). Data from experiments performed in several years with more cultivars and soils with diverse properties will be required to obtain better grounded, more reliable recommendations for farmers.

Keywords: AM fungi inoculation, *Rhizophagus irregularis*, *Glomus mosseae*, winter wheat, grain yield

Introduction

Arbuscular mycorrhizal (AM) fungi are beneficial microbes, ubiquitous in natural and agricultural ecosystems (Pellegrino et al. 2015). Under natural conditions the AM fungi living in symbiosis with plant roots make a substantial contribution to the nutrient (P, N, S, K, Ca, Fe, Cu, Zn) and water uptake of the plants (Smith and Read 2008). Phosphate ions are almost insoluble in soil because of interactions with soil cations, and are very poorly mobile. The traditional model of mycorrhizal function is based on the exchange of phosphate and carbon between plant and fungus (Fitter et al. 2011). In addition to this direct effect, AM fungi also have indirect effects, including the amelioration of the soil structure (Rillig and Mummey 2006; Miller and Jastrow 2010), interactions with other soil-borne microorganisms (Artursson et al. 2006; Veresoglou et al. 2016) and protection against plant pathogens (Hooker et al. 1994; Azcón-Aguilar and Barea 1996). Under intensive

agricultural production conditions AM fungi are of much less significance than in natural conditions. The reduction in the number of plant species found on a given area (e.g. wheat-maize rotation) (Sasvári 2017), the regular disturbance of the soil (Kabir, 2004), the use of mineral fertiliser (Kahiluoto et al. 2001) and the application of fungicides (Jin et al. 2013) all lead to a decline in the number and activity of AM fungi. On areas constantly used for agricultural production the number of AM fungal propagules in the topsoil (0-30 cm) is greatly reduced (Oehl et al. 2005; Posta 2013; Gottshall 2017).

With increasing soil depth, a decrease was found in the percentage of roots colonized by AM fungi, in the number of infective propagules and in the amount of extraradical AM fungi hyphae, but the reducing effect of agronomic practices could not be demonstrated. More remarkably, the AM fungi community composition changed towards deeper soil layers and a surprisingly high species richness was observed even in the deepest soil layers (50-70 cm) examined (Oehl et al. 2005).

Crop plants differ in the extent of their dependence on mycorrhizae for nutrient uptake (Smith and Read 2008). The mycorrhizal dependence of different cultivars of a given species may also be different. Azcon and Ocampo (1981) observed a wide range of dependence on mycorrhizas in experiments with 13 wheat cultivars. Hetrick et al. (1996) investigated the mycorrhiza dependence of ten wheat cultivars, six of which responded positively, while four responded negatively or were nonresponsive to mycorrhizal inoculation. The responses of the individual cultivars were consistent regardless of the inoculum source, suggesting that mycorrhizal responsiveness is an inherited trait rather than a response to individual fungi. Mycorrhizal responsiveness decreased with P fertilisation for cultivars that were dependent on the symbiosis, but it was unaffected by P fertilisation in cultivars that were negatively impacted by the mycorrhizae (Hetrick et al. 1996).

Hetrick et al. (1993) investigated modern wheat cultivars and their ancestors, and suggested that modern breeding practices had reduced dependence on mycorrhizal symbiosis. In contrast Lehman et al. (2012) found no evidence that new crop genotypes lost their ability to respond to mycorrhiza due to agricultural and breeding practices.

The advantages of AM fungi are obvious to crop producers, so the possibility of inoculating soils with these fungi has long been the subject of research. Inoculation with arbuscular mycorrhizal fungi is considered to be a sustainable crop production technology (Azcon and Ocampo 1981).

A number of reviews and meta-analyses have been published, most of which report on the yield increases demonstrated in field crops such as maize and winter wheat (Lekberg and Koide 2005; Hoeksema et al. 2010; Lehmann et al. 2012; Treseder 2013; Pellegrino et al. 2015). Based on these results, the effect of AM fungal inoculation depends on the available nutrient content of the soil, the type of cultivation, the fertilisers applied, the use of plant protection agents (particularly fungicides) and the weather.

The relationship between the available phosphorus (P) content of the soil and the level of mycorrhization has been examined in depth (Hetrick et al. 1996; Lekberg and Koide, 2005; Hoeksema et al. 2010; Treseder 2013; Suriyagoda et al. 2014). It is generally accepted that low available P content in the soil facilitates the development of mycorrhiza while high available P content inhibits it. A few researchers such as Hoeksema et al. (2010) stated that the N fertilisation was a more important predictor of plant responses to AM fungal inoculation than the P content in the soil. Hoeksema et al. (2010) also found that very few studies reported the available soil N and P concentrations or values for other important abiotic factors, such as ambient light or soil water availability. AM fungal communities are also influenced by climatic factors and the success of symbiosis depends on water availability and the temperature during early plant development (Augé 2001).

Most authors of reviews and meta-analyses (Hetrick et al. 1993; Lekberg and Koide 2005; Lehmann et al. 2012; Treseder 2013; Pellegrino et al. 2015) observed the yield-increasing effect of AM fungi. Pellegrino et al. (2015) conducted a well-documented meta-analysis of 38 field trials published between 1975 and 2013, involving a total 333 data, and reported that AM fungal inoculation led to a mean yield increase of 20%. However, the transferral of these results is complicated by the fact that the crop production was extensive (low-input), the grain yields were low (2-5 t ha⁻¹) in most experiments, and only three of the locations tested were in Europe (which means that the climates were different). This is true of all the meta-analyses published in this field.

The size of the experiment is also an important factor. Individual studies have shown that the effects of mycorrhizal fungi on plants are different in the field than in greenhouse or growth chamber experiments (Hoeksema et al. 2010). More specifically, Lekberg and Koide (2005) found that the beneficial effects of AM fungi on plants were smaller in field experiments than in greenhouse or growth

chamber experiments. Despite their scientific importance, field experiments with small plot size may lack agronomic relevance.

The aim of the present work was to determine whether AM fungal inoculation resulted in a grain yield increase in two cultivars with high grain yield potential of winter wheat (*Triticum aestivum*) (Mv Nádor and Genius) under field conditions on 1 ha plots with fertile soil (calci chernozem) using the intensive crop production technology normally applied in Hungary.

Materials and Methods

The field experiment was set up on calcic chernozem soil in the neighbourhood of Nagyhörsök, Hungary. The soil characteristics were as follows: upper level of plasticity according to Arany 43, mean humus content 2.73%, AL-soluble K_2O and P_2O_5 149 mg kg^{-1} and 181 mg kg^{-1} , respectively, $CaCO_3$ 5.05%, $pH_{(KCl)}$ 7.27. The soil analysis was performed in the Soil Protection Laboratory, Velence. The preceding crops on the experimental area were sunflower (2015), maize (2014) and pea (2013).

The climate is continental, with an annual mean temperature of 11 °C and annual mean precipitation of 590 mm. The weather conditions in 2016 were ideal for wheat production.

The AM fungi inoculant used in the experiment was the Aegis Sym Irriga microgranulate manufactured by Italtollina. This contains several AM fungi, principally the *Rhizophagus irregularis* (previously *Glomus intraradices*) and *Glomus mosseae* (syn *Funneliformis mosseae*) species. The inoculant has a concentration of 1,400 spores g^{-1} and the recommended dose is 1–2 $kg ha^{-1}$ (internet 1).

Two winter wheat cultivars were used in the experiment, Mv Nádor and Genius. Mv Nádor (MTA ATK Martonvásár), an early–midseason cultivar, was state registered in 2012. It has a potential yield of 10–11 $t ha^{-1}$, low plant height (60–80 cm), excellent winter hardiness and good flour quality. Genius (Saaten-Union) has a high potential yield in case of extensive and intensive conditions alike. This cultivar has good

frost resistance, winter hardiness and drought tolerance. It has premium milling quality. No data are available on the mycorrhiza susceptibility of either cultivar.

Four treatments (mineral fertiliser + AM fungi inoculant, mineral fertiliser alone, AM fungi inoculant alone, no fertiliser or inoculant) were applied in three replications to both wheat cultivars. The mineral fertiliser dose was 130 $kg ha^{-1}$ N, 78 $kg ha^{-1}$ P_2O_5 and 60 $kg ha^{-1}$ K_2O and the plot size 10,000 m^2 (40×250 m), arranged in a strip-split-plot design. All the plots were given the soil preparation and plant protection normal under farm conditions.

The sunflower crop in the previous year was harvested on 31 Aug. 2015, followed on 1 Sept. by disking and on 14 Sept. by the application of basic potassium fertiliser to the fertilised plots. The seedbed was prepared using a seedbed cultivator on 20 Oct. The AM fungi inoculant was applied on 21 Oct. as recommended by the manufacturer, using plant protection machinery, where the spraying device followed a short disk fitted with a bladed cylinder at a maximum distance of 20 cm. The sowing took place on 26 Oct. The seed was sown within 7 days of inoculation, according to the recommendations of the manufacturer. Ammonium nitrate (34%) was applied to the fertilised plots on 3 Nov. and Nitrosol (30%) on 22 Nov.

Two plant protection treatments were performed. The first, on 5 Apr., consisted of a 0.75 $l ha^{-1}$ dose of the fungicide FalconPro (active ingredients: spiroxamine, tebuconazole and prothioconazole) and a 0.15 $l ha^{-1}$ dose of the herbicide Sekator OD (active ingredients: amidosulfuron and iodosulfuron). On 9 May a 2 $l ha^{-1}$ dose of the fungicide Cherokee (active ingredients: cyproconazole, propiconazole and chlortalonyl) was applied.

Samples were taken with a plot combine at harvest on 12 July 2016. The grain crop were dried to 14% water content.

Root samples were taken at a depth of 5–20 cm, washed, and stored in 0.05% lactoglycerol solution at 4°C. The root colonisation of the AM

fungi was checked under a light microscope after staining the roots with Trypan Blue. The ratio of mycorrhizal roots were measured by the grid line intersection method and were expressed in mycorrhization % (Brundrett 2008).

The statistical evaluation was performed using a paired t-test and multi-factor analysis of variance (ANOVA). Differences were considered to be significant at the $p < 0.05$ level. The differences between the samples means were compared with the value of the LSD with a 95% confidence interval.

Results

The effects of the treatments on the grain yield and mycorrhization of wheat roots were evaluated using analysis of variance for three factors (AM inoculation, mineral fertilisation, wheat cultivar). The grain yields averaged over the factors are given in *Table 1* together with the significance levels of factors. All three factors had a significant effect on the grain yield and (with one exception) interactions between the factors were also significant. The one exception was the interaction between cultivar and AM inoculation.

Mineral fertilisation increased the grain yield from 7.38 to 8.31 t ha⁻¹. AMF inoculation in the same way increased the yield from 7.52 to 8.17 t ha⁻¹. As the interactions between the factors were also significant, the yield averages were also plotted for each treatment (*Table 2*). The two cultivars had different reactions to AM inoculation. The AM inoculation in both cases increased the yield but in a different measure.

Yield of Mv Nádor increased from 6.96 to 8.55 t ha⁻¹, yield of Genius changed from 6.72 to 7.28 t ha⁻¹, the increasing was 123% and 108%, respectively. The reaction to mineral fertilization were also different in case of cultivars. Mineral fertilization increased the yield of Mv Nádor with 132%, while increased the yield of Genius with 108%.

The three factors and their interactions had also significant effects on the mycorrhization of winter wheat roots (*Table 3*). The fertilisation decreased, the AMF inoculation increased the percentages of mycorrhizal roots. The wheat cultivars had also significant effect, Mv Nádor had higher mycorrhization (14.6%) compared to Genius (4.3%).

Discussion

The results of the large (1 ha) plot experiment carried out under farm conditions showed that inoculation with arbuscular mycorrhizal fungi had a detectable yield-enhancing effect on two different cultivar (Mv Nádor, Genius) of winter wheat (*Triticum aestivum*).

Both the winter wheat cultivars tested (Mv Nádor, Genius) are modern, high-yielding cultivars (10–12 t ha⁻¹) recommended for cultivation under intensive conditions. The size of the yield increment, in case of cultivar Mv Nádor, was similar to the ~20% value given in the literature (Pellegrino et al. 2015). The higher yield achieved in response to inoculation with arbuscular mycorrhizal fungi confirms the results of Lehmann et al. (2012) and suggest that

Table 1. Yield averages of winter wheat for each factor (wheat cultivar, mineral fertilisation, AM inoculation). N= 12 (number of replications).

Factor	Yield average (kg ha ⁻¹)		Level of significance
	Mv Nádor	Genius	
Cultivar	8480	7200	0.000
Fertilisation	without 7380	with 8310	0.000
AMF inoculation	without 7520	with 8170	0.000
Cultivar * Fertilisation			0.000
Cultivar * AMF inoculation			0.089
Fertilisation * AMF inoculation			0.002
Cultivar * Fertilisation * AMF inoculation			0.014

Table 2. Yield averages and standard deviations of the wheat cultivars for each fertiliser and AM inoculation treatment. N=3 (number of replications). LSD value for all treatments is 342.

Treatment	Yield averages and standard deviations (kg ha ⁻¹)	
	Mv Nádor	Genius
No fertilisation, no AMF inoculation	6960 (207)	6720 (200)
No fertilisation, AMF inoculation	8550 (190)	7280 (410)
Fertilisation, no AMF inoculation	9160 (430)	7240 (160)
Fertilisation, AMF inoculation	9270 (290)	7570 (220)

even modern wheat cultivars have not lost their ability to form mycorrhizas. The yield averages of the two wheat cultivars differed significantly, the greater yield potential of cultivar Mv Nádor being confirmed in the experiment. Cultivar Genius responded to the AM inoculation in a smaller measure but this cultivar showed smaller yield increasing to the fertilization too. We don't have not enough data to decide that this cultivar has low AM susceptibility or it has limited P uptake potential.

The experimental area has been used for intensive crop production involving replenishment rates of mineral fertiliser (P and K) for several decades. Sasvári (2017) reported that wheat-maize rotation has pronounced detrimental effect on AM fungi. These factors were probably responsible for the fact that the number of arbuscular mycorrhiza-forming fungus propagules had dropped to such an extent that a single mycorrhizal inoculation was able to cause a detectable change in the yield. This was confirmed by the fact that small number

of structures characteristic of mycorrhization (arbuscules, hyphae) could be observed on the roots of plants that were not inoculated (Table 3). Direct spore counting from the soil was not happened.

Data in the literature suggest that the mycorrhization of plants is stimulated if the soil has a low content of available phosphorus, and inhibited by high P content. In the present experiment the available P content in the soil was measured with the method routinely used in Hungary as the quantity of AL-soluble phosphorus, which was found to be 181 mg P₂O₅ kg⁻¹ at the beginning of the experiment. According to the official recommendations (MÉM NAK, 1979) this represents a good phosphorus supply level. As the meta-analyses, quoted above in the introduction, generally either give no data on the available phosphorus content of the soil or measure it using a different method (other than AL-solubility), this complicates a comparison of the results. On the other hand

Table 3. Mycorrhization of winter wheat for each factor (wheat cultivar, mineral fertilisation, AM inoculation). N= 12 (number of replications).

Factor	Mycorrhization (%)		Level of significance
	Mv Nádor	Genius	
Cultivar	14.6	4.3	0.000
Fertilisation	without 18.9	with 0.03	0.000
AMF inoculation	without 0.85	with 18.1	0.000
Cultivar * Fertilisation			0.000
Cultivar * AMF inoculation			0.000
Fertilisation * AMF inoculation			0.000
Cultivar * Fertilisation * AMF inoculation			0.000

beside the soil P content it is necessary to take plant P demand into consideration.

The yield-enhancing effect of AM fungi inoculation could only be detected in treatments given no mineral fertiliser (*Table 2*). The mineral fertiliser treatments involved a total of 130 kg N ha⁻¹, 78 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹ active agents, based on the nutrient management calculations usually employed on the farm. As NPK mineral fertiliser was applied in the present work, therefore it is not possible to separate the effects of nitrogen and phosphorus fertiliser on mycorrhization. There is agreement in the literature that the application of phosphorus fertiliser to agricultural crops reduces the level of mycorrhization of the crops, as the crop itself is able to take up the necessary quantity of phosphorus and is not dependent on a fungal partner. Very few papers reached other conclusions.

One such is that of Cozzolino et al. (2013), who demonstrated the yield-enhancing effect of NP and NPK fertiliser in maize plants inoculated with mycorrhizal fungi. This could be due to the fact that the soil had very different characteristics to that used in the present experiment.

Our results support the idea usually accepted in the literature, that mineral fertilisation (mainly P) decreased the efficiency of AM inoculation.

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Conclusions and recommendations

The results and the conclusions drawn from them were based on the data of a single year (2016). Our research showed that AM fungi inoculation of winter wheat in field conditions can be an effective agronomic practice, although its economic profitability should still be questioned. Data from experiments performed in several years with more cultivars and soils with diverse properties will be required to obtain more reliable recommendations for farmers. The harmonisation of the production technology and the AM fungi inoculation is important for efficient crop production. Changes in the production technology could produce more favourable conditions for the fungi, making the AM fungi inoculation more successful. Many papers have reported on the favourable effects of reduced disturbance/ploughing, the use of vegetation cover and the incorporation of organic matter.

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