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Research Article

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Arbuscular mycorrhizal fungi inoculation of winter wheat in an intensive crop producing farm

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Abstract
Arbuscular mycorrhizal fungi (AMF) inoculation represents a valid tool for improving crop yield, primarily in the case of extensive crop production. The conditions of intensive crop production inhibit the growth of AMF and decrease the plant root mycorrhization. To clarify the possibilities of AMF inoculation under intensive farm conditions, a two-year field experiment was set up. The effect of AMF inoculation and mineral fertilization (130 kg N ha^{-1}, 78 kg P_2O_5 ha^{-1}, 60 kg K_2O ha^{-1}) were investigated on the crop yield, crop P content and root mycorrhization of two modern winter wheat (Triticum aestivum L.) cultivars. The soil was Chernozem with calcareous precipitates (WRB classification: Calcic Chernozem). The AMF inoculum contained reproductive units of Rhizophagus irregularis and Glomus mosseae. The whole experimental area was 32 ha and cultivated according to the usual intensive crop production of the farm. Both AMF inoculation and mineral fertilizer treatment had a significant effect on crop yield and root mycorrhization. The yield-enhancing effect of AMF inoculation was only observed in plots without mineral fertilizer and depended on the interaction of the year and the cultivar. AMF inoculation did not affect the P content of the crop. In the dry year the effect of AMF inoculation was cultivar dependent. AMF inoculation increases the yield to a smaller extent than the mineral fertilization. Our results show that AMF inoculation without fertilization can be profitable under intensive farming conditions.

Keywords: AMF Inoculation, Winter Wheat, Intensive Cultivation, Farm Scale Experiment.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the majority (over 72%) of vascular plants, including many crops [1]. AMF take up water, phosphorus, and other nutrients for plants and receive some of the organic carbon fixed during plant photosynthesis in return. AMF-plant symbiosis has a great importance in plant nutrient uptake, especially in adverse conditions (e. g. drought). In addition to their role in nutrient uptake, several other effects of AMF symbiosis have been demonstrated. They also play an important role in maintaining a favourable soil structure and in the ability of plants to defend against pathogens [2-7].

In parallel with the spread of sustainable agricultural practices, the use of AMFs to replace or reduce fertilizer doses and improve soil structure emerged early. The potential benefits of AMF inoculation have been of great interest to the end-users and the companies. Basiru et al. (2021) listed 68 commercially available mycorrhizal products from 28 manufacturers across Europe, America and Asia [8]. There are numerous reports on the positive effects of AMF inoculation on crop yield or plant biomass. Using the published experimental results of AMF inoculation in field experiments, several review articles and meta-analyses have been reported. Meta-analyses have shown that AMF inoculation can increase aboveground biomass or crop yield. Plants with arbuscular mycorrhiza often show increased nutrient, especially P, concentrations [9-14]. However, inoculation by AMF does not always lead to improved plant performance. Even under controlled greenhouse conditions, failure of colonisation is common and in cases of successful colonisation, effects range from negative, negligible to significant yield gains [15]. The use of reviews and meta-analyses in crop production practice is hampered by the results obtained under different geographical, climatic, cultivation and farming conditions. For example, in the meta-analyses of Pellegrino et al. (2015), only 3 out of all (38) sampling sites were in Europe.

To be useful for the plant, AMF must be present in or introduced into the soil, and secondly, they must be protected. At present, most of the intensive cropping systems used worldwide do not protect

AMFs and do not favour mycorrhizal development. Therefore, to maintain a rich AMF community in the soil must inoculate yearly, which means additional costs. Seed coating technology can offer a solution to reduce this cost of inoculation [16]. Conversion to less intensive agricultural management systems, such as organic bio-based farming and no-tillage, may reduce the negative impacts of conventional management on AMF [17,18]. The minimal or zero tillage methods used instead of conservative cultivation have been shown to increase the inoculum potential of soil, making them well suited to AMF inoculation procedures [17-19]. Reducing the dose of fertilizer also helps AMF to grow [19-22]. Mycorrhizal colonisation of plants is negatively correlated with P concentration in soil [19]. The good nutrient (especially P) supply of the plant reduces the need for AMF, so P fertilization generally reduces the effectiveness of AMF inoculation [23]. Soil N supply or N fertilization has less influence on inoculation [24,25].

It is difficult to integrate the AMF inoculation into current intensive agricultural systems, and it is also questionable to what extent it is worth changing standard cultivation methods (e. g. reducing disturbance and using lower doses of fertilizers and pesticides), given the need to maintain high yields. Manipulation or completely changing farming systems in favour of AMF is only economically viable if there is clear evidence that AMF contributes positively to yields [26]. But temporary cessation of mineral fertilization can sometimes occur on the intensive crop producing farms and in such cases the AMF inoculation can help to maintain high yield averages. In case of extensive cultivation the AMF inoculation together with moderate mineral fertilization increased the crop yield even in a conventional crop producing system [27].

Among the biotic factors that modify the effect of AMF on host yields, the properties of the host plant are critical. The mycorrhizal dependence varies not only between crops but also between cultivars [28,29]. The high-yielding crops widely used today are the product of artificial selection, typically under high nutrient conditions [12]. Hetrick et al. (1993), in a greenhouse study of 20 wheat cultivars, found that cultivars released before 1950 benefited more consistently from AMF inoculation in terms of biomass, whereas the response of cultivars released after 1950 was more variable [30]. However, later studies have shown different results [31,32,12]. The origin of cultivars received much less attention. It is not known whether the mycorrhizal responsiveness of locally bred cultivars differs from that of globally used cultivars [31,12].

Intensive agricultural crop production does not favour the growth of AMF. Due to the use of fertilizers, the plant does not need the nutrients taken up by the fungi, the disturbance caused by tillage disrupts the hyphal network, and the fungicides used can directly damage the fungi [20,19]. Because of these adverse effects, the number and diversity of AMF propagules is lower in agricultural soils than in natural ecosystems [24,33,18]. The AMF population in the soil can only be maintained by annual inoculation. There are few reports available on the effect of AMF inoculation under intensive plant cultivation conditions due to the reasons mentioned above, To the best of our knowledge, such an experiment in large-scale plots has not been described in the literature. The aim of our study was to investigate the effect of AMF inoculation on winter wheat yield, P content and root mycorrhization at farm-scale (1 ha plot size, conditions of intensive crop production). Apart from the application of AMF inoculation, agricultural practices used on the farm have not changed. Our objectives were: (1) Do AMF inoculations have a yield-enhancing effect in an intensive crop production farm? (2) Do AMF inoculations have an additive effect with NPK fertilization? (3) Do modern winter wheat cultivars with different origin respond differently to AMF inoculation?

2. Materials and Methods

The field experiment was set up on *Calcic Chernozem* (WRB classification) in the vicinity of Nagyhörcsök, Hungary (GPS coordinates: 46.891286, 18.519395). The soil texture was loam, characteristics were as follows: mean organic matter content 1.61%, 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, Hungary.

The climate is continental, with an annual mean temperature of 11 ^{o}C and annual mean precipitation of 590 mm. The weather conditions in 2016 were ideal for wheat production (417 mm precipitation in the growing period) but the year 2017 was dry (245 mm precipitation). The AMF inoculant used in the experiment was the Aegis Sym Irriga microgranulate manufactured by Italpollina (Italpollina s.p.a. Verona, Italy). This contains several AM fungi, principally *the Rhizophagus irregularis and Glomus mosseae* species. The inoculant has a concentration of 1,400 spore g^{-1} and applied in the recommended dose (2 kg ha^{-1}).

Two modern winter wheat (*Triticum aestivum L.*) cultivars were used in the experiment, Mv Nador and Genius. Mv Nador (MTA ATK Martonvásár, Hungary), an early–midseason cultivar bred for local conditions. Cultivar Genius (Saaten-Union, Germany) widely used in Europe, it has a high potential yield in case of extensive and intensive conditions alike. No data is available on the mycorrhiza susceptibility of either cultivar.

An intensive crop producing farm (their averages of winter wheat yield are between 7.5-9.0 t ha^{-1}) provided the experimental area. They used conservative farming practices which are widespread in Hungary, with ploughing, crop rotations (containing sunflower, winter wheat, corn, and pea) and plant protection measures. The preceding crops in the experimental area were sunflower. Four treatments, NPK+ AMF+ (mineral fertilization and AMF inoculation), NPK+ AMF- (mineral fertilization alone), NPK- AMF+ (AMF inoculation alone) and NPK- AMF- (without mineral fertilization and without AMF inoculation) were applied in three replications to both wheat cultivars. The mineral fertilizer dose was 130 kg ha^{-1} N, 78 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O. This mineral fertilizer dose corresponds to the dose usually applied by the farmers. The plot size was 10,000 m² (40×250 m), arranged in a strip-split-plot design. The whole experimental area was 32,000 m^2, all plots were cultivated in the same way, according to the usual farm practices.

Samples were taken with a plot combine at harvest on July 2016 and 2017. 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 % [39].

All statistical analysis were performed using the SPSS 27 software package. Mean values are given with their standard error in each table. Plant parameters were compared among the treatments by using multi-factor analysis of variance (ANOVA) followed by the Duncan post-test. The effects size (pEta2) was used to statistically estimate the magnitude of a given effect.

3. Results

The set-up of the experiment allowed us to determine the significance level and the effect size (pEta2) of the different factors (AMF inoculation, mineral fertilization, year, and cultivar) on the crop yield, root mycorrhizal colonisation, and crop P content. The calculated effect sizes and significance levels of factors are shown in Table 1. The effect of all four factors was significant on crop yield and root mycorrhizal colonisation. Crop P content was only significantly affected by fertilization. The R^2 values of ANOVA model for the crop yield, root mycorrhizal colonisation, and crop P content 0.939, 0.985 and 0.400, respectively. The four factors well explain the variations of crop yield and root mycorrhizal infection, while the low R^2 values of the model of crop P content indicate that factors other than those examined should be considered.

Table 1: The Effects Sizes (Peta^2) and Significance Levels of Year, Cultivar, AMF Inoculation and Mineral Fertilization on the Crop Yield, Root Mycorrhizal Colonisation and Crop P Content.

Factor	Crop yield	Root mycorrhizal colonisation	Crop P content
AMF inoculation	0.449 **	0.939 **	0.018
NPK fertilization	0.809 **	0.958 **	0.141 *
Year	0.869 **	0.719 **	0.047
Cultivar	0.680 **	0.683 **	0.046

The symbols *, **, and *** show statistical significance at the 0.05, 0.01, and 0.001 level, respectively.

The effect of AMF inoculation and fertilization treatment on crop yield is shown in Table 2. The average yield of fertilized treatments was 7649 kg ha^{-1}, and that of non-fertilized treatments was 6102 kg ha^{-1}. The average yield of AMF inoculated treatments was 7215 kg ha^{-1} and that of the non-inoculated was 6536 kg ha^{-1}. The weather in the two experimental years was very different. The first year had an average good weather for winter wheat, while the second year was dry and droughty. Due to the different weather and the significant effect of year and cultivar interaction, the effects of treatments on yields are shown in Table 2 separately for each year. In the first year, the average yield of fertilized treatments was 8310 kg ha^{-1}, and that of non-fertilized treatments was 7377 kg ha^{-1}. The mean of AMF inoculated treatments was 8167 kg ha^{-1} and that of non-inoculated ones was 7520 kg ha^{-1}. In the second (dry) year, the average yield of fertilized treatments was 6988 kg ha^{-1}, and that of non-fertilized treatments was 4828 kg ha^{-1}. The average of AMF inoculated treatments was 6264 kg ha^{-1} and that of non-inoculated ones was 5552 kg ha^{-1}. In the dry year the yield increase due to AMF inoculation was significant only for the Mv Nador cultivar. The two cultivars responded similarly to fertilization: in the first year both cultivars had significant yield increases, albeit to different degrees: 31.7% and 7.7% (Mv Nador and Genius). In the second year the yield increases were 65.3% and 25.5% (Mv Nador and Genius), but the difference was significant only in the case of Mv Nador.

Table 2: Crop Yield Averages of the Winter Wheat Cultivars for Each Treatment, Separately (N=3) and Both Years Together (N=6).

Treatment	Mv Nador	Genius	Both cultivars
	(kg ha^{-1})		
first year			
AMF- NPK-	6955 a	6723 a	6839 a
AMF- NPK+	9157 c	7244 b	8201 b
AMF+ NPK-	8549 b	7279 b	7914 b
AMF+ NPK+	9270 c	7568 b	8419 b
first year total	*8483*	*7204*	*7843*
second year			
AMF- NPK-	4539	4508 a	4523 a

AMF- NPK+	7504	5657 ab	6580 b
AMF+ NPK-	5568	4697 a	5133 a
AMF+ NPK+	7848	6942 b	7395 b
second year total	*6365*	*5451*	*5908*
both years together			
AMF- NPK-	5457 a	5615 a	5681 a
AMF- NPK+	8331 c	6450 b	7391 c
AMF+ NPK-	7059 b	5989 ab	6524 b
AMF+ NPK+	8559 c	7255 c	7907 d
both years total	*7424*	*6327*	*6875*

The lowercase letters after the averages per column show the results of the Duncan tests between treatments.

The effect of AMF inoculation and mineral fertilization on root mycorrhization is shown in Table 3. For all data, the mean root mycorrhizal infection was 0.4% in the fertilized and 27.6% in the non-fertilized treatments; the average was 25.1% in inoculated and 2.9% in non-inoculated treatments. AMF inoculation with fertilization together did not increase the mycorrhization of roots. Due to the different weather years and the significant effect of year and cultivar factor on roots mycorrhization, the effects of treatments on yields are shown separately by year (Table 3). There was a significant difference in the degree of root mycorrhization between the two years. In the favourable weather of first year the mycorrhization of roots was 9.4%, while in the second (dry) year it was 18.5%. The mycorrhization of both cultivars increased in the second year compared to the previous year. Both cultivars gave similar reactions when AMF inoculation and fertilizer treatment were applied together. In the first year the average mycorrhization of roots in fertilized treatments was 0.02% and that of non-fertilized treatments was 18.9%. The mean of AMF inoculated treatments was 18.1% and that of the non-inoculated was 0.8%. In the second (dry) year the average mycorrhization of fertilized treatments was 0.8%, and that of non-fertilized treatments was 36.3%. The mean of AMF inoculated treatments was 32.2% and that of the non-inoculated was 4.9%.

Table 3: Root Mycorrhization Averages of the Wheat Cultivars for Each Treatment, Separately (n=3) and in Both Years (n=6) Together.

Treatment	Mv Nador	Genius	Both cultivars
	(%)		
first year			
AMF- NPK-	3.0 a	0.2 a	1.5 a
AMF- NPK+	0.0 a	0.0 a	0.0 a
AMF+ NPK-	55.3 b	16.7 b	36.0 b
AMF+ NPK+	0.0 a	0.0 a	0.0 a
first year total	*14.6*	*4.3*	*9.4*
second year			
AMF- NPK-	14.0 b	5.7 b	9.8 b
AMF- NPK+	0.0 a	0.0 a	0.0 a
AMF+ NPK-	67.0 c	58.7 c	62.8 c
AMF+ NPK+	2.3 a	0.7 a	1.5 a
second year total	*20.8*	*16.3*	*18.5*
both years			
AMF- NPK-	8.5 b	2.8 a	5.7 b
AMF- NPK+	0.0 a	0.0 a	0.0 a
AMF+ NPK-	61.2 c	37.7 b	49.4 c
AMF+ NPK+	1.2 a	0.3 a	0.8 a
both years total	*17.7*	*10.2*	*14.0*

The lowercase letters after the averages per column show the results of the Duncan tests between treatments.

Taking together the data of the two years, the P content of the crop was significantly influenced by fertilization only. The interactions of the year with other factors are not significant, and therefore, unlike the previous crop yield and root mycorrhization, the data for the two years are reported only in aggregate (Table 4). Regarding the crop P content, the two cultivars responded differently. Mv Nador reacted positively to the fertilizer treatment, the crop P content increased, while the crop P content of the Genius cultivar did not change. AMF inoculation had no significant effect on the crop P content of either cultivar. The R^2 value of ANOVA model was low, which means that the crop P content of the crop was also significantly influenced by factors that could not be considered in ANOVA.

Table 4: Crop P Content Averages of the Wheat Cultivars for Each Fertilizer and AMF Inoculation Treatment, in Both Years (N=6).

Treatment	Mv Nador	Genius	Both cultivars
	(g kg^{-1})		
AMF- NPK-	3.33 a	4.17 a	3.75a
AMF- NPK+	4.50 b	4.17 a	4.33a
AMF+ NPK-	3.50 a	4.33 a	3.92a
AMF+ NPK+	4.50 b	4.50 a	4.50b
total	*3.96*	*4.29*	*4.13*

The lowercase letters after the averages per column show the results of the Duncan tests between treatments.

4. Discussion

Our experiment was set up on a farm that has been intensively cultivating crops for a long time (at least for 20 years). For economical reason the conventional agronomical practices were not modified other than the introduction of AMF inoculation.

4.1. Effects of AMF Inoculation

AMF inoculation increased the crop yield of winter wheat in our experiment, in accordance with the meta-analyses of other authors [11,12]. The rate of increase in crop yield in AMF inoculated treatments was on average 10% compared to the non-inoculated treatments. In the above-mentioned meta-analyses, the values of increase were between 16% and 20%. But there was a significant number of extensively managed sites in the database of the meta-analyses mentioned, so it is particularly important to highlight that in our experiment the yield increase was obtained under intensive farming conditions. AMF inoculation alone (without fertilization) increased the mycorrhization of roots. This increase in mycorrhization is consistent with the previous study, although other authors have found that mycorrhization does not show a strong relationship with the usefulness of symbiosis [34,35]. However, the AMF inoculation together with fertilizer application did not increase the crop yield and the root mycorrhization. Crop P content was not significantly increased by AMF inoculation. This is in contrast to previous reports that have generally found an increase in the crop P content in the case of AMF inoculation [35,11,14].

4.2. Effects of Mineral Fertilization

The applied NPK fertilization with or without AMF inoculation increased the yield and P content of the crop in both years, but reduced the mycorrhization of the roots. The decrease in mycorrhization due to P fertilization is known from the literature, therefore it is doubtful whether NPK fertilization should be used in combination with AMF inoculation [17,36,37,12]. Low P fertilization or adequate N/P ratio fertilization does not necessarily reduce mycorrhization [24,25]. Soil-based P supply and N/P ratio may be the factors that can decide this issue from a practical point of view. The mycorrhization of roots in the un-inoculated control soils was low in our experiments, probably because the number of AMF propagules was low in the soil. Extraradical hyphae are thought to be the main source of inoculum in soils [38]. These hyphae are sensitive to tillage and our experimental area has been cultivated intensively for decades. The inoculum potential of the soil was not determined in our experiment. Among factors investigated, fertilization was the only one, which modified the P content of the crop. Years, cultivars, and AMF inoculation had no effect on P content. But the effect size of fertilization on P content was low, and these factors explained only a small fraction of the total variance (Table 1). Based on these results, other factors (e. g. light intensity, soil P supply, its temporal change and spatial distribution) can determine the P content of the crop. The AMF inoculation has no additive effect when used in combination with NPK fertilization.

4.3. Effects of Cultivars

Both winter wheat cultivars used in our experiment, Mv Nador and Genius, are new, high-potential modern cultivars. However, their genetic backgrounds are different, because of the different breeding companies. The extent of differences in mycorrhization between new and older cultivars is a matter of debate in the literature. Because of fungal and plant genotype interactions, there are differences in fungal responsiveness between the modern cultivars, also [28]. In our experiments the mycorrhization of the two new cultivars with different origin was different. The locally bred cultivar (Mv Nador) was more susceptible to AM fungi, but based on our results there is not necessarily a relationship between the yield and mycorrhization. In the dry year, the mycorrhization of cultivar Mv Nador increased to a lesser extent than that of cultivar Genius, but its average yield increased. It probably adapted to the less favourable conditions in other ways. The increase in mycorrhization measured in cultivar Genius did not manifest in crop yield in the dry year, and from an agronomic point of view, AMF inoculation had no effect on that cultivar. Our study confirmed the important role of plant genotypes in the interaction between plant and fungus, and that the development of symbiosis is also highly dependent on abiotic factors (e. g. weather).

4.4. Effects of the Weather

The weather in the two experimental years was very different and this had different effects on crop yield and mycorrhization of roots. Mycorrhization values were higher in the dry year, but this was not reflected in the crop yield, which was lower in all cases than in the previous, normal year. AMF inoculation may have helped the plants to grow and develop, but this was only manifested in crop yields for the cultivar Mv Nador. In the case of the cultivar Genius, mycorrhization increased as a result of AMF inoculation, but this did not affect crop yield. To confirm our results several years of experiment would be needed, because root colonization often has shown no consistency from one year to another [25].

5. Conclusion

AM fungi inoculation caused a measurable yield increase compared to un-inoculated and unfertilized treatment on a farm with intensive crop production, with little change in the usual agricultural practices of the farm. It is important to note that the nutrient supply of the soil was good as, prior to our experiments, the soil was continuously given a high dose of NPK fertilizer.

Together with NPK fertilization, AMF inoculation had no additional effect on the crop yield, root mycorrhization or crop P content. NPK fertilization increases yield to a greater extent than AMF inoculation. In the dry weather year, the effect of AMF inoculation was cultivar dependent. It has been concluded from our experimental data that NPK fertilization is a safer way to achieve a high yield in drought conditions compared to AMF inoculation.

For maintaining the fungi in the soil, originating from AMF inoculation, it would be important to introduce “fungus-friendly”, sustainable cultivation methods, to maintain a plant-efficient AMF community in the soil instead of the annual AMF inoculation. Organic farming and no-till managements may be the possible solution for the AMF, but it is economically questionable for the farmers. Our results show that AMF inoculation without fertilization can be profitable under intensive farming conditions.

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