

## THE EFFECTS OF N-FERTILIZATION LEVELS AND ROOT MYCORRHIZAL COLONIZATION ON PLANT-GROWTH AND GRAIN YIELD IN DURUM WHEAT

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### Abstract

In a two-year study (2002-2003), mycorrhizal treatments in conjunction with three nitrogen fertilization levels of 0, 50, 100 kg of N/ha were tested on durum wheat in continuous cropping. The study took place in the Province of Foggia, a semi-arid area of the Mediterranean Basin in southern Italy, with silty-clay soil classified as Typic Chromoxerert. Seeds of cultivar *Simeto* were inoculated at sowing, and a completely randomized block design with three replications with and without inoculation was adopted. Plant samples were taken during the vegetative cycle of the durum wheat, and the root mycorrhizal colonization was examined. In all treatments, the percentage of colonised root length was significantly higher in the inoculated plants than it was in the control. The plants inoculated and fertilized with 50 kg N/ha showed a significant increase in grain yield.

Additional Keywords: *Triticum durum*, mycorrhizal, nitrogen

### Introduction

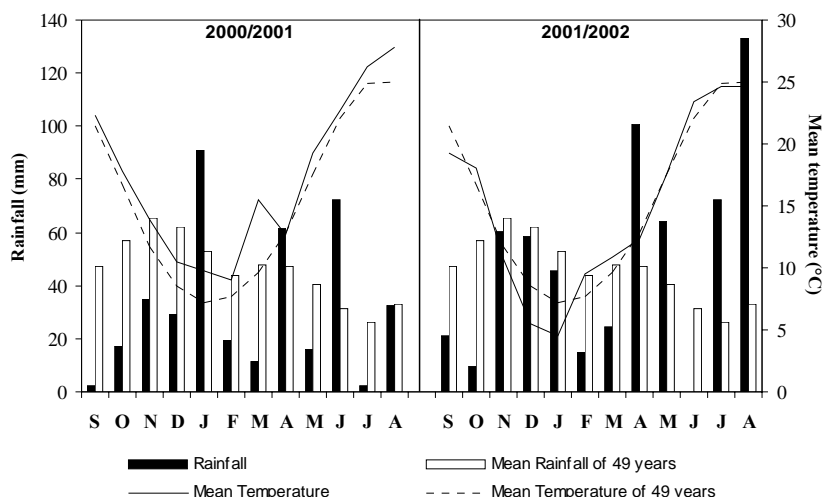
The need to preserve soil fertility and protect the environment from detrimental agronomic techniques has brought about a revision of productive systems in agriculture. Recently, the employment of beneficial microorganisms has gained popularity (Pearson V. and Read D.J., 1973; Giovanetti and Gianinazzi-Pearson, 1994; Perotti *et. al.*, 1996). The mutualistic association between roots and mycorrhizal fungi can improve a plant's nutritional state since it facilitates the absorption of the main elements in the soil (N, P, K), increases the volume of soil explored by the root system, improves the plant's resistance to some diseases, and increases its production of dry matter (Barber, 1995; Smith and Read, 1997; Giovanetti and Sbrana, 1998). The effects of mycorrhizas seem to increase in non-optimal nutritional conditions. In environments with scarce precipitation, the presence of these fungi can make the plants more resistant to water stress and strengthen their ability to use the nutrients naturally occurring in the soil (Staddon *et. al.*, 2002; Koide and Dickie, 2002).

Low levels of precipitation are common in lower Mediterranean Basin areas such as the Apulian plain known as the *Tavoliere Pugliese* in southern Italy, a region where cereal crops dominate. The rainfall levels in this area limit the effect of fertilization on durum wheat crops. The results of studies on the application of mycorrhizas in cereal crops vary and appear to be frequently influenced by the environmental conditions in the trial area (Baon *et. al.*, 1993; Thompson, 1990; Hetric *et. al.*, 1995). To better understand these interactions, the Istituto Sperimentale Agronomico of Bari and the Dipartimento di Biologia e Patologia Vegetale at the University of Bari carried out a study on the effects of mycorrhizas in durum wheat cultivation. The goal was to evaluate the possibility of improving a plant's nutritional state through alternative treatments or in association with selected dosages of chemical fertilizers.

### Materials and methods

#### *Field site and climate*

The study was carried out in 2002 and 2003 on durum wheat cultivar *Simeto* in continuous cropping in the Apulian plain *Tavoliere*, a cereal growing area of southern Italy. The soil of the experimental plots, which are part of the Istituto Sperimentale Agronomico, is a deep alluvial vertisol, silty-clay textured. It is classified as Typic Chromoxerert by the Soil Taxonomy-USDA (Gee *et al.*, 1986) and has an acceptable agronomic fertility. The main analytic parameters are: pH on 1:2 soil water suspension = 8.12; organic matter by the Walkley-Black method = 2.32%; available P by Olsen and Sommers method = 77 mg/kg, exchangeable K by Thomas method = 1685 mg/kg. The climate in the area is hot and dry in the summer and relatively cold in the winter. It is classified as "accentuated thermo-Mediterranean" on the FAO-UNESCO Bioclimatic Maps of the Mediterranean area. During the two-year study, the temperatures as well as the rainfall levels were both within the norm for the area. The precipitation was 455 mm in the first year and 670 mm in the second (Figure 1). The difference, with respect to the average precipitation over the last 49 years, was -95 mm in the first year and +118 mm in the second. During the most important months for growing wheat in this area, November through May, the rainfall levels peaked at 262 mm in the first year and 368 mm in the second.



**Figure 1. Monthly temperature and rainfall at the experimental field compared to the mean values over the previous 49 years**

#### *Experimental design and treatments*

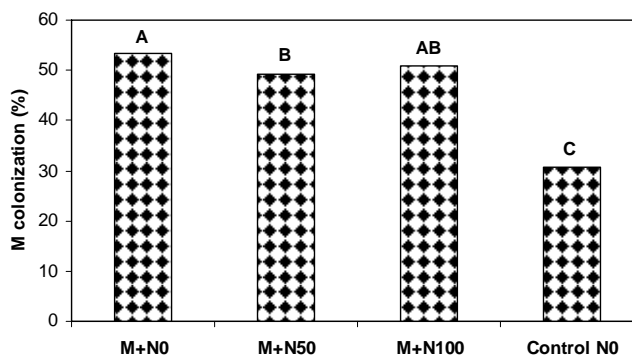
A completely randomised block design with three replications was used, with 50m<sup>2</sup> plots (5x10m) as elementary experimental units. Six treatments were compared: 0, 50 and 100 Kg of N/ha (N0, N50, N100) with and without mycorrhizal application to the seeds. The pre-established quantities of fertilizer were administered in two equal applications at the beginning of tillage and shooting. The inoculation took place just before sowing the seeds; a fungal compound in the ratio of 50 kg per 2 t of seeds per hectare gram per kg of seed was thoroughly mixed into the seed bulk by turning it several times, up to reaching a uniform mycelium dispersal. The fungus preparation was made of topsoil and peat containing bacteria (*Pseudomonas* spp., *Bacillus* spp.) at the approximate concentration of 1x10<sup>7</sup> CFU/g (Colony Forming Unit) and about 50% of spores and mycelia of endo-mycorrhizal fungi of the genus *Glomus* (*Glomus mosseae* (Nicolson & Gedermann) Gedermann & Trappe, *G. caledonium* (Nicolson & Gedermann) Gedermann & Trappe, *G. viscosum* (Nicolson), *G. intraradices* Schenck & Smith, *G. coronatur* Giovannetti).

#### *Analysis*

Samples of the soil and of the mycorrhizal fungus were collected and analysed to determine the conditions before sowing. For each m<sup>2</sup>, 350 seeds were sown. During the cultivation cycle, the main vegetative phases (sprouting, tillage, shooting, flowering and grain maturation) were recorded. A 10-plant sample from each elementary plot was collected at the end of tillering, stem extension (last leaf just visible) and flowering. These samples were removed with their roots and most of the attached soil to analyse the extent of mycorrhiza formation. After washing the roots free of soil, representative fresh samples were cleared for 10 minutes in KOH (10% w/v solution) in a pressure chamber at 121°C, and were stained with trypan blue (5% w/v in lactoglycerol solution, i.e. 1:1:1 lactic acid-glycerol-water) (Phillips and Haymann, 1970). The percentage of mycorrhizal root length was assessed using the grid-line intersect method (Brundrett *et al.*, 1996; Giovannetti and Mosse, 1980; Newman, 1966). At harvest, the yield and the main biological parameters were recorded. In the second year, each plot underwent the same treatment as in the previous year. The experimental data were analysed statistically by a one-way analysis of variance after ARCSIN transformation and the means were separated by Duncan's test (SAS, 1993).

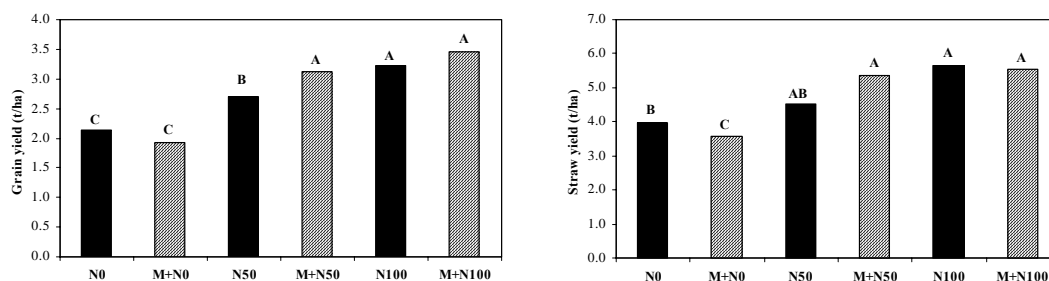
#### **Results and Discussion**

The mycorrhizal fungi demonstrated a good ability to interact with the root system of wheat. Within the treatments with mycorrhiza, the percentage of colonised root length was slightly higher without fertilization than with fertilization (N50 and N100) (Figure 2). This result could indicate that the colonisation of roots by fungi suffers a slight decline in the presence of nitrogen fertilizer. However, this phenomenon does not have a negative effect on the plant yield as will be presented below.



**Figure 2. Mycorrhizal colonization of durum wheat plant roots in percentage of the root length. Inoculation with mycorrhizal fungi (M) and fertilization levels (N0 = 0, N50 = 50 and N100 = 100 Kg N/ha). Values followed by the different letter are significantly different  $P \leq 0.05$  (S.N.K. test)**

A comparison of the average grain yields (Figure 3) of the nitrogen-fertilized plots with those having had mycorrhizas added to them reveals that the latter had a productivity increase of 0.15 t/ha. Indeed, the mycorrhizas positively influenced the grain yield only in the presence of nitrogen applications. In the plots without fertilization, the yield from the treatment with mycorrhiza even showed a slight yet not significant decline in productivity of 0.20 t/ha. In combination with N50 fertilisation, the positive effect of the treatment with the bio activator on grain yield was evident and demonstrated a significant difference of 0.41 t/ha with respect to the equivalent plot in which only nitrogen was administered. In combination with N100 fertilisation, the production increased in comparison to the lower nitrogen treatment and the positive influence of the mycorrhizas continued. However, in this case the increase in grain yield was 0.23 t/ha and, thus, was not significant with respect to the equivalent N100 treatment without the seed fungal application. The straw yield shows trends similar to those of the grain yield. In absence of fertilization, there was a significant effect of reduction in the plot treated with mycorrhizas. Likewise, the combination of mycorrhiza+fertilization effectively increased the yield compared with the treatments without fertilization (Figure 3).



**Figure 3. Two year means of durum wheat grain yield (above) and straw yield (below) for the different N-fertilization levels (N0 = 0, N50 = 50 and N100 = 100 kg N/ha) and for the same N-fertilization levels in combination with mycorrhizal fungi (M). Values with different letters are significantly different at  $P \leq 0.05$  (S.N.K. test)**

Among the biometric parameters (Table 1), the 1000-seed weight was higher in the mycorrhiza-treated plots. Additionally, there was a significant difference between the results in these plots and those in the plots with only nitrogen added. An exception to this, however, can be seen in the N50 plot which had a 1000-seed weight similar to the value from the corresponding treatment plus the bio activator (M+N50). The combination of mycorrhiza+fertilization acted positively in reducing the percentage of yellow berry seeds. The decreases were 38% and 28% in the N50 and N100 plots, respectively. This result is of great importance since it represents an improvement in the quality of the grains. Instead, no influence was noted on the percentage of stunted kernels in the two-year trial. For the parameter of plant height, the only significant difference was the higher value in the plot with mycorrhiza and N100 fertilisation. In comparison with the other treatments, this increased value seems to be the consequence of the greater nitrogen fertilization.

**Table 1. Means of the yield components of the durum wheat for the N-fertilization levels and N-fertilization levels with mycorrhizal fungi**

	1000 seeds weight (g)	Yellow berry (%)	Stunted kernel (%)	Plant height (cm)
<b>Nitrogen (N)*</b>				
N0	48.84 <sup>B</sup>	33.41 <sup>A</sup>	3.00	60.00 <sup>C</sup>
N50	51.34 <sup>A</sup>	27.66 <sup>B</sup>	3.33	65.77 <sup>AB</sup>
N100	49.86 <sup>AB</sup>	13.08 <sup>C</sup>	4.50	66.58 <sup>AB</sup>
<i>Mean</i>	<i>50.01</i>	<i>24.72</i>	<i>3.61</i>	<i>64.11</i>
<b>Mycorrhizal f. (M) + Nitrogen (N)</b>				
M+N0	51.31 <sup>A</sup>	35.50 <sup>A</sup>	3.17	60.48 <sup>CD</sup>
M+N50	51.80 <sup>A</sup>	19.91 <sup>C</sup>	3.00	66.08 <sup>AB</sup>
M+N100	51.53 <sup>A</sup>	10.16 <sup>D</sup>	4.00	68.33 <sup>A</sup>
<i>Mean</i>	<i>51.54</i>	<i>21.86</i>	<i>3.39</i>	<i>64.96</i>

\* N0, N50 and N100 = 0, 50 and 100 kg of N/ha respectively.

Values with different letters in columns are significantly different at  $P \leq 0.05$  (S.N.K. test).

### Conclusions

The treatment with mycorrhizal fungi interacted positively with nitrogen fertilization. In absence of fertilization, even though the percentage of wheat roots colonised with fungus was greater in the plots treated with mycorrhizas, the grain yield, the 1000-seeds weight, plant height as well as the incidence of yellow berries and stunted kernels remained constant, while the straw yield decreased. In the presence of mycorrhizal fungi and nitrogen fertilization, the trends for grain and straw yield as well as those for yellow berry improved significantly with respect to both controls and the corresponding treatments with nitrogen only. These increases occurred without changes in other parameters such as the 1000-seeds weight or plant height. The positive effects observed can be interpreted as having the potential for improving the durum wheat cultivation techniques in semi-arid environments. The best increases were obtained from the combination of mycorrhizas and 50 kg of N/ha. It can be concluded that mycorrhizas improve the ability of durum wheat to take advantage of nitrogen fertilization, thus, having a positive effect on both crop yield and the environment.

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