Single or mix mycorrhizal fungi inoculum? The potential role of different mycorrhizal fungi

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Abstract

Seeded plants of several grass species were grown in a mix or single culture for a 3-year period, at a site situated inside the Taxiarchis University Forest (Chalkidiki, northern Greece) with sub Mediterranean climate. One hundred 10-litres in volume containers were filled with mix soil from B and C horizons with sandy loam texture and low available phosphorus. The soil parent material was para-gneiss. Ten replicated treatments were inoculated with Gigaspora margarita BEG 34, ten with Glomus intraradices BEG 144, ten with Acoulospora longulata BEG 8, ten with a mixture of the BEG isolates used and ten with a mixture of indigenous species. Plant tissue analysis suggested that accelerated growth occurred after mycorrhizal application. However, significant variations on growth were observed at different fungal treatments and seasons. It is suggested that variations on growth could be explained by differences on the ability to access phosphorus and the limited phosphate source at the soil used, the inter-fungal interactions and the functional compatibility with the host plant.

Key words: mycorrhizal symbiosis, soil properties

Introduction

The majority of the terrestrial plants form an obligatory symbiosis with soil borne fungi, forming arbuscular mycorrhizal (AMF) symbiosis which is the most abundant mycorrhizal among plants. There is a large literature about the plant fungal interactions, as far as the plant physiology concerns. There is also an adequate of knowledge about the mechanisms of the symbiosis. Despite the large literature on the AMF symbiosis little is known about the role of AMF when applied in open field experiments, particularly in the Mediterranean regions. Productivity of the Mediterranean lands is closely depended upon the soil properties and the climatic conditions. The fungal symbiont allows the plants to withstand harsh soil environment and colonise sites of low nutrient availability. The mycorrhizal fungi could expand the rhizospheric zone to a vast area, forming a mycorrhizosphere. The efficacy of the mycorrhizosphere is determined from both the plant and the soil conditions. The soil properties, along with the plants could affect the chain of events from the fungal spore germination to root colonisation. Soil pH, temperature, moisture, light, aeration, inorganic compounds and the presence of bacteria are among those affecting AMF spore germination

(Garbaye 1994). AMF have been found in soils with pH 2.7 to 9.2 (Killham 1994). Different AMFs could have their optimum at different soil conditions. In particular, Acaulospora spp. have been reported widely in acidic soils (Nicolson and Schenk 1979, Young et al. 1985, Morton 1986); Glomus spp. were found in soils of pH>5.5 but were absent in soils of pH 4.5 and lower (Wang et al. 1993); Gigaspora spp. have been reported in more acidic soils than Glomus spp. (Clark 1997). Thus, soil properties could initially affect the fungal biodiversity, since it is possible that different fungal species could have different symbiotic compatibility optimum at different soil properties. Such variations may result in a different plant growth response, when plants are in symbiosis with different fungal species or with different mix of AMFs. Such differences upon growth responses could determine plant biodiversity in natural lands. Significant efforts have been made recently to apply AMF commercially at various field applications. However, the provenance of the fungal species or genera was overlooked. Considering the evidence of AMF functional compatibility along with differences on the host AMF dependency, and by that, observed plant diversity could be determined by the existing fungal biodiversity; the research about the application of AMF in various field trials is necessary (Van der Heijden et al. 1998).

Mediterranean soils are heavily disturbed and often the surface soil is removed by erosion. Phosphate bioavailability could be very limited under such harsh soil conditions. The host plants grown in grasslands are usually of high mycorrhizal dependency, particularly at limited soil phosphate availability conditions. Differences occurring on growth performance should be related to the symbiont compatibility not only with the host plant but also with the soil environment. The present research investigates the potential use of various single fungal species inoculum along with some mix inoculum cultures, upon the grassland production when the plant dependency on AMF is high.

Materials and methods

One hundred 10-litres in volume containers were filed with fine soil material originated from a C and B-soil horizon over paragneiss. The soil pH was 5 and the extractable P 6.9 mg/kg. The soil material was sprayed with VAPAM in order to minimise any microbiological activity. Ten containers received seed mix of Poa, Cynodon, Plantago and Agrostis respectively.

Five containers from each plant treatment were inoculated with single BEG AMF isolate (Glomus intraradices BEG 144, Gigaspora margarita BEG 34, Acoulospora longula BEG 8), or with a mix of the five selected BEG isolates, or with a mix of indigenous AMFs. For the period of three years, all plant

material was harvested at early July and late September at the end of the growth period. Dry weight determination and a complete plant tissue analysis were conducted to the plant material collected at each harvest. Soil analysis was conducted to both sites were the C% and the organic matter was estimated (Nelson and Sommers 1982), the organic N%, the extractable P (Olsen and Sommers 1982). Plant tissue analysis was also conducted and N%, P, Mg, Ca, K, Na were measured. Mycorrhizal colonisation was estimated with the grid line intersect method. Randomly selected plants were used in order to measure the effects of the indigenous AMF population.

Results

Mycorrhizal colonisation resulted variations at the plant growth after inculcation with different AMF fungi originated from the BEG or with a mix of the selective BEG isolates or with a mix of indigenous fungi (Figure 1). Inoculation with indigenous fungi has a better result on plant growth up to 78% at the early stages of growth of the experiment. Inoculations with G. intraradices however, enhance the growth of plants significantly better three years after the initial inoculation. Colonisation with Acoulospora resulted to the minimal or no beneficial growth. Plant tissue analysis suggests that the effect of AMF inoculation clearly enchase plant growth, except from those inoculated by A. longula. Finally the data suggesting that different fungal treatment show different phosphate levels.

Discussion

Data analysis clearly shows the beneficial effect on plant dry weight after mycorrhizal inoculation with selective AMF isolates. The plant response varies in relation to the isolate used. The source of this variation needs further consideration. It is clearly that the effectiveness of different AMF isolate variation is depended upon the soil conditions, simply because different fungi can compensate the soil environment differently. The increased efficacy of plants inoculated with indigenous AMF species at the early years of the experiment was gradually reduced since changes occurred at the soil used as substrate. The extractable P of the soil used as a substrate to non-inoculated control plants was reduced to approximately 50% one year after the beginning of the experiment. The plants used the available forms of P at the substrate used, by that, all the extractable P values were dropped. The P at the given pH of the substrate material used is immobile and not available to the plant roots. Colonisation with AMF resulted in rather constant extractable P levels to the soil. However, it changes gradually while colonisation by AMF resulted in a significant reduction of extractable soil P,

particularly after inoculation with G. intraradices. Changes of the extractable P level occurred gradually since the fungal symbionts used the available P in plant favour. Indigenous AMF were more efficient to use the soil resources at the beginning of the experiment. However this was not the case at later time where plants with more biomass and P at the tissue were in symbiosis with G. intraradices. The ability of plant roots in symbiosis with G. intraradices was high enough to uptake rock phosphate. The ability of G. intraradices to uptake rock phosphate efficiently was reported previously at different conditions and different hosts (Duponnois et al. 2005). Presumably, the indigenous mycorrhizal species were in harmony with the plant species used by providing them soil resources at rates easily compensate by the plant. The finding presented here clearly suggesting that the indigenous species can enhance growth from the early stages of plant growth. However, inoculation with an AMF isolate with an aggressive character could improve possibly at later stage the plant growth (Ouahmene et al. 2007).

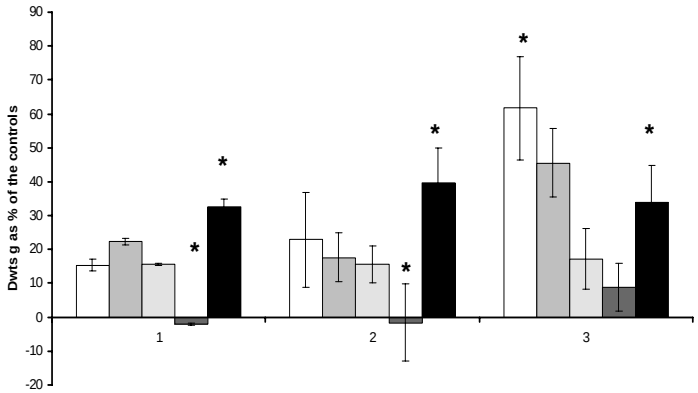

Figure 1. Effects on plant dry weights after inoculation with different arbuscular mycorrhizal fungi at the three years of the experiment. Glomus intraradices (empty), Gigaspora margarita (lined), Acaulospora longula (squered), mix of BEG isolates (sphere), indigenous AMF (filled). Bars are standard error. Data points marked with an asterisk are not significantly different from each other ($P < 0.05$).

The effect on plant growth of BEG's mix inoculums is also important. Clearly, the fungal species used were probably in competition for resources. Competition for carbon among different AMF species after colonisation of

the same root system has been previously reported (citation). The competition of the different AMF species often results to a reduced host growth, as the plant fails to support the increased carbon fungal demands, particularly after inoculation with Glomus and Gigaspora spp. Similar interactions were possibly responsible for the relative reduced growth of the plants inoculated with the mix BEG inoculums.

Inoculation with Gigaspora margarita and Acoulospora longulata had no significant effect on plant dry weight. Possibly these AMF species didn't compensate the soil conditions and although formed symbiosis with the plants used their effects on growth were not different from the uninoculated control plants. The increased P nutrition of plants inoculated with G. margarita or A. longulata was not enough to promote growth against the controls. However, as the P level at the plants inoculated with the G. margarita or A. longulata did not change significantly at the 3-year period of the experiment. It is believed that gradually will overcome the controls simply because they will have a permanent access to the soil P, while the values of the plant tissue phosphate in the controls were gradually reduced.

Clearly the P uptake improved not only by the AMF effect on the inorganic soil. Mycorrhizal altered the soil conditions in favour of bacterial population due to the increase of sugar exudation to the soil (Hooker et al. 2007). Those conditions could change the bacterial population resulting to changes at the P uptake from inorganic sources (Mayer and Linderman 1986).

Mycorrhizal application should take under consideration the fungal species used and the soil conditions along with the nature of the agricultural product. The outcome of mycorrhizal applications on the field should take under consideration all the contributing partners to the symbiosis development, the soil conditions, the host plant and the inoculum used at the application.

References

Clark R B. 1997. Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. *Plant Soil* 192: 15-22.

Duponnois R; Colombek A; Hien V; Thioulouse J. 2005. Fungus Glomus intraradices and rock phosphate amendment in fluence plant growth and microbal activity in the rhizosphere of Acacia holosericea. *Soil biology and Biochemistry* 37, 1460-1468.

Engel R; Boller T; Wiemken A; Sanders I R. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72.

Garbaye J. 1994. Tansley Review No 76. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol*. 128: 197-210.

Hooker J E; Piatti P; Cheshire M V; Watson C A. 2007. Polysaccharites and monosaccharlites in the hyphopsere of the arbuscular mycorrhizal fungi Glomus E3 and Glomus tenue. *Soil Biology and Biochemistry* 39, 680-683.

Killham K. 1994. Soil ecology, Cambridge University press, 254p.

Meyer J R; Linderman R G. 1986. Response of subterranean clover to dua inoculation with vesicular- arbuscular fungi and a plant growth-promoting bacterium, Pseudomonas putida. *Soil Biol. Biochem.* 18: 185-190.

Morton J B. 1986. Three new species of Acaulospora (Endogonaceae) from high aluminium, low pH soils in West Virginia. *Mycologia* 78: 641-648.

Nelson D W; Sommers L E. 1982. Total carbon, organic carbon and organic matter requirement. In Methods of Soil Analysis. Part 2. A L. Page, ed. Amer. Soc. of Agronomy and Soil Sci.Soc. of America, Madison, Wisconsin, USA, pp:539-577.

Nicolson T N; Schenck N C. 1979. Endogonaceous mycorrhizal endophytes in Florida. *Mycologia* 71: 178- 198.

Olsen S R; Sommers L E. 1982. Phosphorous. In: Methods of Soil Analysis. Part 2.A.L. Page, ed. Amer. Soc. Of Agronomy and Soil Sci.Soc. of America, Madison, Wisconsin, USA, pp: 403-427.

Siqueira J Q; Hubbell D H; Mahmud A W. 1984. Effect of liming on spore germination, germ tube growth and root colonisation by vesicular-arbuscular mycorrhizal fungi. *Plant Soil* 76: 115-124.

Van der Heijden M G A; Klironomos J N; Ursic M; Moutoglis P; Streitwolf-Engel R; Boller T; Wiemken A; Sanders I R. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72

Wang G M; Stribley D P; Tinker P B; Walker C. 1993. Effects of pH on arbuscular mycorrhiza. I. Field observations on the long-term liming experiments at Rothamstead, Woburn. *New Phytol*. 124: 465-472.

Young J L; Davis E A; Rose S L. 1985. Endomycorrhizal fungi in breeder wheats and triticale cultivars field- grown on fertile soil. *Agronomie J.* 77: 219-224.