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# Effect of inoculation with soil yeasts on mycorrhizal symbiosis of maize

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## Summary

Interactions between arbuscular mycorrhizal fungi (AMF) and soil yeasts were studied in a pot experiment conducted in substrate from a spoil bank. Maize was inoculated with AMF (*Glomus intraradices* BEG140 and *Glomus mosseae* BEG95), three soil yeast species (*Candida sake*, *Cryptococcus aerius* and *Williopsis californica*) and combinations of these microorganisms. In plants inoculated with yeasts alone, only *Candida sake* increased maize growth while the dual inoculation of plants with *Glomus intraradices* and any of the three yeasts showed positive effects on shoot biomass. Presence of soil yeasts did not significantly affect mycorrhizal colonisation of maize roots but negatively affected the length of the AMF extraradical mycelium (ERM). Soil yeast numbers were significantly influenced by AMF with both positive and negative effects observed. It can be concluded that dual inoculation of maize with yeasts and AMF resulted in increased shoot biomass depending on the combination of yeast species and AMF isolate.


## Introduction

Soil inoculation with microorganisms such as arbuscular mycorrhizal fungi (AMF) (Smith and Read, 1997) to establish a microflora beneficial to plant nutrition and health may play an important role in revegetation programmes. Rhizosphere microorganisms can produce exudates, which affect the growth of plants and microorganisms present in the soil (Jeffries et al., 2003). Microbial interactions are thus significant as they may modify the relationship between plants and AMF (Fracchia et al., 2004). Yeasts are a common component of the rhizosphere in all geographic zones (Slávikova

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and Vadkertiová, 2003); however, there is little knowledge of their in nutrient cycling (Slávikova et al., 2002) and their interaction with other soil microorganisms. Only a few studies have investigated AMF interactions with soil yeasts (Fracchia et al., 2003; Sampedro et al., 2004). The aim of this study was to determine (i) the effect of dual inoculation with soil yeasts and AMF on biomass production of maize in a spoil-bank substrate, (ii) the effect of yeasts on the colonisation of maize roots by AMF and (iii) the length of the extraradical mycelium (ERM) of AMF in the soil.

Three yeasts species – *Candida sake*, *Cryptococcus aerius* and *Williopsis californica* – were isolated according to Wuczkowski et al. (2003) from agricultural soils near Vienna, Austria, where maize is a common crop in the crop rotation. The AMF were isolated in the Czech Republic, *Glomus mosseae* BEG95 from a coalmine spoil bank (N-Bohemian coal basin,) and *Glomus intraradices* BEG140 from a sedimentation pond of pyrite smelter (Chvaletice). The substrate for the pot experiment was loess ($pH_{KCl} = 7.5$, $C/N = 1.54$, $P = 24.8\,mg\,kg^{-1}$, $Mg = 1156\,mg\,kg^{-1}$ and $Ca = 2324\,mg\,kg^{-1}$) collected from a spoil bank of the Vršany coalmine (N-Bohemian coal basin), mixed with perlite 1:1 (v/v) and sterilised by $\gamma$-radiation. The soil yeasts were propagated according to Sampedro et al. (2004). The soil was inoculated with yeast in aqueous suspension 2 weeks before mycorrhizal inoculation and transplantation of plants (Fracchia et al., 2003). The AMF were applied as a liquid inoculum. The control treatment was supplied with the same quantity of heat-sterilised mixed inoculum plus inoculum filtrate to arrive at a similar quantity of organic matter and bacterial conditions for all treatments. Furthermore, filtrate from the original soil containing indigenous soil microflora was added to all treatments. The seeds of maize (*Zea mays* L. cv. TATO) were pre-germinated on heat-sterilised moistened sand and uniform seedlings were transplanted, one plant per pot. The factorial design of the experiment included both control treatments (without yeasts and AMF; see Table 1) in five replicates. Shoot and root biomass of each plant were determined after 12 weeks. Root samples were stained according to Koske and Gemma (1989) and mycorrhizal colonisation was evaluated according to a modified method of McGonigle et al. (1990). The total ERM length was assessed using a modified membrane filtration technique (Jakobsen et al., 1992). The ERM extraction was also performed in the non-inoculated control treatment to determine the background of dead fungal hyphae in the original soil. The evaluation of the soil yeast populations during the experiment was performed according to Fracchia et al. (2003).

The data were subjected to one-way and two-way ANOVA after tests for the homogeneity of variance and the normal distribution of the residues were conducted. Any significant differences were

**Table 1.** Effect of inoculation with AMF (*Glomus intraradices* and *Glomus mosseae*) and soil yeasts (*Candida sake*, *Cryptococcus aerius* and *Williopsis californica*) on shoot biomass and root colonisation of maize and on the length of fungal ERM

| AMF | Soil yeast | Shoot biomass (g dry matter) | Root colonisation (%) | ERM length (mm $g^{-1}$ dry soil) |
|---|---|---|---|---|
| Non-inoculated | Non-inoculated | 2.1 ± 0.2 b | 0 ± 0 ns | 407 ± 146 ns |
| | *C. sake* | 2.9 ± 0.5 a | 0 ± 0 ns | 430 ± 95 ns |
| | *C. aerius* | 2.4 ± 0.1 b | 0 ± 0 ns | 357 ± 103 ns |
| | *W. californica* | 2.3 ± 0.1 b | 0 ± 0 ns | 360 ± 164 ns |
| *G. intraradices* | Non-inoculated | 2.1 ± 0.5 b | 69.4 ± 5.1 ns | 3153 ± 1047 a |
| | *C. sake* | 2.8 ± 0.1 a | 65.2 ± 4.1 ns | 1597 ± 842 b |
| | *C. aerius* | 2.5 ± 0.1 a | 66.8 ± 3.9 ns | 2443 ± 239 ab |
| | *W. californica* | 2.5 ± 0.2 a | 60.2 ± 5.2 ns | 1473 ± 247 b |
| *G. mosseae* | Non-inoculated | 2.2 ± 0.2 ab | 44.6 ± 3.6 ns | 4053 ± 358 a |
| | *C. sake* | 2.4 ± 0.2 a | 46.4 ± 5.9 ns | 3387 ± 668 a |
| | *C. aerius* | 2.4 ± 0.4 a | 44.2 ± 3.6 ns | 2610 ± 152 b |
| | *W. californica* | 1.8 ± 0.2 b | 44.4 ± 5.1 ns | 2360 ± 679 b |
| AMF (1) | | * | *** | *** |
| Soil yeast (2) | | *** | ns | *** |
| 1 × 2 | | * | ns | ** |

Data are means of five replicates, different letters within each AMF inoculation treatment indicate significant differences according to Duncan's multiple range test ($P < 0.05$). Effects of factors according to ANOVA: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant.

tested using Duncan's Multiple Range test ($P<0.05$).

While soil yeasts increased shoot biomass of maize, AMF sometimes slightly reduced maize growth (Table 1). There was also a significant interaction between the effect of yeast and mycorrhizal inoculation on plant growth. In non-mycorrhizal controls only *Candida sake* significantly increased growth of maize while positive growth effects on shoot biomass was observed for all three yeasts in plants inoculated with *Glomus intraradices*. In treatments inoculated with *Glomus mosseae*, the addition of *Candida sake* and *Cryptococcus aerius* resulted in better plant growth as compared to the addition of *W. californica* (Table 1). Root biomass was not affected either by inoculation with soil yeasts or AMF (data not shown). *Glomus intraradices* developed significantly higher levels of mycorrhizal colonisation of maize roots in comparison with *Glomus mosseae* (Table 1). The presence of yeasts had no significant effect on mycorrhizal colonisation of maize roots; however, negative effects of yeasts on the length of ERM was observed in most treatments (Table 1). The presence of *Candida sake* and *W. californica* significantly decreased ERM length of *Glomus intraradices*. Inoculation with *Cryptococcus aerius* and *W. californica* caused significant reduction of ERM development in *Glomus mosseae*. In general, there were no significant differences in CFU numbers between individual species of soil yeasts (Table 2). However, yeast numbers were significantly influenced by AMF; both positive and negative effects were observed. For *Candida sake*, significantly lower CFU numbers were found in treatments inoculated with *Glomus mosseae* as compared to inoculation with *Glomus intraradices* and the non-inoculated control. In contrast, *Glomus mosseae* positively affected CFU numbers of *W. californi*ca. Population of *Cryptococcus aerius* was significantly reduced in presence of *Glomus mosseae* as compared to *Glomus intraradices* (Table 2).

Numerous studies have revealed that interactions between soil microorganisms and AMF are important for plant growth (Azcón-Aguilar et al., 2002; Barea et al., 2002). Our results showed that inoculation with soil yeasts and AMF can significantly affect shoot dry weight of maize; moreover, we found specific effects of certain combinations of mycorrhizal inoculation and yeast species on plant biomass. In plants not inoculated with AMF, only *Candida sake* increased plant growth compared to plants inoculated with *Glomus intraradices* where all three yeasts showed positive effects on shoot dry weight. Somewhat similar results were obtained by Bhowmik and Singh (2004). In their experiment, inoculation of *Chloris guyana* with the yeast *Saccharomyces cerevisiae* alone did not affect plant growth. However, dual inoculation with the yeast and the AMF *Glomus mosseae* resulted in significant increase of plant biomass. Sampedro et al. (2004) also reported increased growth of soybean

**Table 2.** Effect of inoculation with AMF (*Glomus intraradices* and *Glomus mosseae*) on CFU numbers of the soil yeasts (*Candida sake, Cryptococcus aerius* and *Williopsis californica*)

| Soil yeast | AMF | Number of CFU ($g^{-1}$ $_{dry\ soil}$) |
|---|---|---|
| Non-inoculated | Non-inoculated | 40 ± 55 ns |
| | G. intraradices | 40 ± 55 ns |
| | G. mosseae | 40 ± 55 ns |
| *C. sake* | Non-inoculated | 3020 ± 1472 a |
| | G. intraradices | 2880 ± 420 a |
| | *G. mosseae* | 780 ± 240 b |
| *C. aerius* | Non-inoculated | 1740 ± 319 ab |
| | G. intraradices | 3140 ± 2090 a |
| | *G. mosseae* | 480 ± 320 b |
| *W. californica* | Non-inoculated | 1360 ± 430 b |
| | *G. intraradices* | 1280 ± 460 b |
| | *G. mosseae* | 2780 ± 500 a |
| Soil yeast (1) | | *** |
| AMF (2) | | ** |
| 1 × 2 | | *** |

Data are means of five replicates, different letters within each yeast inoculation treatment indicate significant differences according to Duncan's multiple range test ($P<0.05$). Effects of factors according to ANOVA: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, ns = not significant.

inoculated with the AMF *Glomus mosseae* and the yeast *Rhodotorula mucilaginosa*. Fracchia et al. (2003) showed positive growth response of soybean growing in symbiosis with *Glomus mosseae* and red clover associated with *Gigaspora rosea* when co-inoculated with soil yeasts *R. mucilaginosa*, *Cryptococcus laurentii* or *Saccharomyces kunashirensis*. However, the two latter studies were not carried out in a fully factorial design since treatments inoculated with soil yeasts only (without AMF) were not included. For this reason, it was not possible to separate the effect of soil yeasts and the AMF on plant growth. The plant benefit of dual inoculation in our experiment occurred presumably due to the uptake of nutrients, probably released during the decomposition of yeast biomass or nutrient transformation by the soil yeasts (Falih and Wainwright, 1995). The subsequent transport to the plants by the ERM hyphae resulted in increased plant growth as proposed by Linderman (1992). Soil yeasts in our experiment did not affect mycorrhizal colonisation of maize but significantly decreased the length of fungal ERM. This is in contrast with results of other authors, who found an increase in mycorrhizal colonisation of plant roots and stimulation of hyphal growth from spores in the presence of various yeasts (Fracchia et al., 2003; Sampedro et al., 2004). The inhibition of fungal ERM growth observed in our experiment could be, therefore, attributed to the observed reduction of shoot biomass in the mycorrhizal treatments without soil yeasts. By transferring photoassimilates to their mycorrhizal roots, plants incur reduced shoot biomass production. Some role in these relationships can probably be ascribed to water-soluble yeast exudates, because positive effects on AMF development were also induced by the addition of soil yeasts exudates instead of living yeast cells (Fracchia et al., 2003; Sampedro et al., 2004). In contrast to ERM, which can be attacked by soil organisms, the intraradical phase of the fungus is protected against microbial antagonism (Azcón-Aguilar and Barea, 1992). Probably for that reason, no negative effects of soil yeasts on root colonisation were observed in our experiment. AMF isolates in our study showed different effects on populations of soil yeasts. While *G. intararadices* did not affect CFU numbers of any yeast, *Glomus mosseae* either decreased or increased yeast population size depending on the yeast species. These results are contrary to observations of Sampedro et al. (2004), who found no effect of the presence of the AMF *Glomus mosseae* on populations of *R. mucilaginosa*, *Cryptococcus laurentii* and *S. kunashirensis* in the rhizosphere of soybean. Fracchia et al. (2003) observed similar CFU numbers of *R. mucilaginosa* in rhizosphere of soybean inoculated with *Glomus mosseae* and in rhizosphere of red clover inoculated with *Gigaspora rosea*. AMF can significantly influence the microflora in the rhizosphere directly through fungal exudates or indirectly through altering root exudation patterns (Linderman, 1992). Several studies revealed that although some species or groups of microorganisms can be increased in the mycorrhizosphere of AMF, different AMF might exert differing effects on selected microbial groups (Ames et al., 1984; Secilia and Bagyaraj, 1987). Moreover, mutual relationships between soil microbiota and the AMF can be modulated by factors such as soil pH, nutrient content, organic matter, moisture and other soil properties (Azcón-Aguilar and Barea, 1992; Linderman, 1992). Although the soil yeasts and AMF in our experiment generally did not exhibit positive mutual relationships, dual inoculation of maize with yeasts and AMF resulted in increased shoot biomass depending on the combination of yeast species and AMF isolate. Further research is needed to select compatible and efficient combinations of AMF and beneficial microorganisms for successful use in revegetation of disturbed ecosystems.

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