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Managing arbuscular mycorrhizal fungi for bioprotection: Mn toxicity

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ABSTRACT

We investigated whether an intact extraradical mycelium (ERM) is more effective than other forms of propagule from indigenous arbuscular mycorrhizal fungi (AMF) in providing protection against stress to a host plant. The response of wheat (*Triticum aestivum* L.) to Mn toxicity was studied in a two-phase greenhouse experiment. In Phase 1, four Mn tolerant species from the natural vegetation, ranging from strongly mycotrophic to non- or weakly mycotrophic, were grown to develop different amounts of ERM. Wheat was then planted (Phase 2) with the ERM fragmented by sieving (Disturbed Treatment) or kept intact with no prior soil disturbance (Undisturbed Treatment). The growth of wheat was doubled by earlier and faster mycorrhizal colonization (AC) in the presence of an intact ERM at planting. There was a positive correlation between plant growth and the reduction of Mn and enhancement of P and S uptake into shoots. However, the growth of plants in undisturbed soil was significantly affected by the ERM developer species, which was not explained by differences in AC. Colonization starting from an intact ERM greatly enhanced the potential of AMF for protection against Mn toxicity. However, the degree of protection depended on the plant previously grown to develop the ERM, suggesting that there may be functional diversity within the ERM developed by mycotrophic plants of the natural vegetation.

1. Introduction

Many benefits can accrue to plants from their association with arbuscular mycorrhizal fungi (AMF), depending on the environmental conditions (Gupta et al., 2000). The contribution from arbuscular mycorrhiza (AM) is greater under marginal biotic or abiotic conditions than in commercial agriculture. In natural ecosystems, the most important role of AM may be in bioprotection rather than in the acquisition of nutrients (Garg and Chandel, 2010).

The role of AMF in protecting their host against pathogens is well documented for several combinations of cultivated plants and fungal or nematode diseases (Harrier and Watson, 2004). Similarly, there is good evidence for beneficial effects of AMF in soils with different abiotic stresses, such as Al, Mn and heavy metal toxicity (Yano and Takaki, 2005; Nogueira et al., 2007; Hall, 2002). The diversity of AMF may influence the outcomes of these interactions for both biotic (Thygesen et al., 2004; Lax et al., 2011), and abiotic stresses (Kothari et al., 1991; Oliveira et al., 2006). However, the majority of investigations fail to consider the richness of indigenous AM fungal communities (Whipps, 2004; Wehner et al., 2010). Nevertheless, such communities seem to exhibit a greater potential for protection (Tchabi et al., 2010). The great diversity of the microbial population present in the mycorrhizosphere (Toljander et al., 2007) also plays an important role in protecting against biotic (Neeraj and Singh, 2011; Siasou et al., 2009) and abiotic stresses (Nogueira and Cardoso, 2002). Despite the complexity of all these interactions, it is recognized that a well-established AM is crucial for an adequate degree of protection (Khaosaad et al., 2007; Garg and Chandel, 2010). The mycorrhiza must be created and be well-established before contact with the stressor, to achieve a high level of protection (Rufyikiri et al., 2000; Petit and Gubler, 2006; Nogales et al., 2009). However, under field conditions, when the stressor is already present in the soil, the role of AMF in protection is challenged by the time required to achieve an adequate level of AMF colonization, together with the cost associated with the large-scale application of commercial inoculum (Sikora et al., 2008).

The extraradical mycelium (ERM) of mycorrhizas might be important for enhancing the roles of AM under field conditions. ERM is particularly efficacious as a propagule that even plant species usually not hosting mycorrhizal fungi can be colonized (Püschel et al., 2007). Root colonization from ERM starts earlier and develops faster than from other types of propagule (Martins and

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Read, 1997; Fairchild and Miller, 1988). Additionally, ERM formed by indigenous AMF will encompass the functional diversity of the local fungal population and its associated microbes, which is expected to be greater than that of any introduced commercial inoculum. Under agricultural systems ERM can develop on tolerant crops, cover crops (Kabir and Koide, 2000) or natural vegetation that grows before seeding susceptible crops (Brito et al., 2011). However, when different plants and fungi are grown together, AMF growth and species composition is host specific (Hart et al., 2003). Therefore the plants used to develop the ERM before the crop to be protected, could influence the final outcome. The benefits to nutrient acquisition, especially accumulation of P, following AM colonization starting from ERM are well documented (Fairchild and Miller, 1988; Goss & de Varennes, 2002) but no information was found in the literature about impacts on the mechanisms underpinning protection.

Manganese toxicity is associated with acid soils and with other soils that have undergone temporary waterlogging, resulting in reduced soil oxygen sufficiently to convert sparingly soluble Mn oxides to the more soluble Mn^{2+} form. For cereals, concentrations of Mn in the shoots above 100 mg kg^{-1} are considered high (Walsh and Beaton, 1973). AMF species and the associated microbial population seem to have different abilities to protect the plants against Mn toxicity (Posta et al., 1994; Nogueira and Cardoso, 2002). The mechanisms of protection are not fully understood, but a reduction of Mn absorption in AM colonized plants has been reported (Nogueira et al., 2004, 2007). These authors also suggested a possible interaction with enhanced P absorption, which could increase plant tolerance to the internal concentration of Mn. According to Goss et al., (1992) the expression of Mn toxicity can also be related to Mg availability, with ratios of Mg:Mn in the soil solution above 100 allowing unimpaired growth of wheat. Therefore another possible protection mechanism of the AMF on Mn toxicity could be through an increased acquisition of Mg. However, several authors have reported that Mg accumulation is unaffected by mycorrhiza development (Marschner and Dell, 1994; Alloush and Clark, 2001; Cardoso et al., 2003).

We hypothesized that AM formation starting from a well-established intact ERM from indigenous AMF and its associated microbial population, would provide more efficacious protection to sensitive plants because AM colonization will start earlier and develops faster than colonization started from other sources of propagule, especially if developed on plants tolerant to the stressor. To test this hypothesis, we chose to work on a soil presenting Mn toxicity in sensitive plants. Toxic ions are continuously present in the soil, so susceptible plants require rapid protection after germination.

2. Material and methods

2.1. Soil properties and characteristics

A sandy loam Eutric Cambisol, known to give rise to Mn toxicity in wheat (Goss and Carvalho, 1992), was used in a two-phase pot experiment under a controlled environment. The soil was collected in the autumn from the top 20 cm of the headland for a long-term natural pasture at Mitra Farm of the University of Evora, Alentejo, Portugal (38° 32′ N; 08° 00′ W). Basic fertility assessment showed that the air-dried and sieved (4 mm) soil contained 1.5 mg P kg^{-1} (Olsen), 28.2 mg K kg^{-1}, 0.4 mg $N–NO_3$ kg^{-1}, 22.6 mg Mn kg^{-1} (DTPA – diethylenetriaminepentaacetic acid), 11 mg OM (organic matter) g^{-1} and had a pH (water) of 6.0. There were 180 (most probable number – MPN) viable AMF propagules per gram of dry soil, consistent with AM formation not being limited by available propagules (Al-Karaki and Clark, 1999).

2.2. Treatments and experimental protocols

Two factors were studied: ERM developer species, grown in the first phase of the experiment, and the integrity of the ERM, present at the beginning of the second phase. Wheat was grown as the test plant in the second phase of the experiment (Experiment 1) because of its susceptibility to Mn toxicity and importance worldwide. The experiment was then repeated (Experiment 2).

In Phase 1, of each experiment, ERM developer species, *Silene gallica* L., *Rumex bucephalophorus* L., *Lolium rigidum* L. and *Ornithopus compressus* L., were planted in 8 L pots. These species are widespread throughout temperate regions, including on soils with Mn toxicity, and exhibit different levels of mycotrophy, ranging from highly mycotrophic (*Lolium* and *Ornithopus*) to very weakly (*Rumex*) or non-mycotrophic (*Silene*, negative control). An additional control treatment, in which No-Plants were allowed to grow prior to the wheat, was included to evaluate AMF colonization of wheat predominantly from spores. This treatment also acted as a control to discriminate between the effects of developer species on growth of wheat through changes in the availability of Mg, Mn, P and S due to plant absorption. The pots from this treatment were packed and maintained similarly to the pots with developer plants. Hereafter this control treatment is referred as “No-Plants”. The ERM developer plants grew for 7 weeks to allow a good establishment of mycorrhiza and the development of an abundant ERM on the mycotrophic plants. Any weeds that emerged were removed daily from the pots by hand. Pots were kept in a greenhouse and watered approximately to field capacity (0.17 g g^{-1}) by weight. The temperature control of the greenhouse only allowed regulation of the maximum temperature, which was set at 30 °C. Minimum and maximum air temperatures were recorded on a daily basis.

At the end of Phase 1, all the developer plants were killed by herbicide (6 mL per pot of a solution containing 1.3 g L^{-1} of glyphosate as Roundup® Supra™). To ensure that the herbicide was not a factor in the experiment, it was also applied to the pots of the No-Plants treatment.

In Phase 2 of each experiment, the level of integrity of the ERM (Factor 2) was achieved by mechanical disturbance of the soil (fragmented ERM, Disturbed treatment) in half of the pots, with the remaining pots being left undisturbed (intact ERM, Undisturbed treatment). In the Disturbed treatment, the shoots of the ERM developer plants were excised and the soil was removed from each pot as two layers of approximately 0.2 m depth and passed separately through a 4 mm sieve. All root material separated on the sieve was cut into 2 cm long segments and mixed into the soil of the appropriate layer. Soil was repacked in the pots and arranged in the same two layers. Shoot material was left intact on the soil surface. In the Undisturbed treatment, the shoots of the ERM developer were also excised and left on the soil surface to ensure that transfer of assimilates from shoots was not a factor in the experiment.

Wheat (*Triticum aestivum* L., var. Ardila) was chosen as the test host plant. Six wheat seedlings were planted, thinned to three plants after ten days. Wheat plants grew for 540 degree-days (base temperature 0 °C), corresponding to 21 days in Experiment 1 and 35 days in Experiment 2. Live ERM developer plants were never present during the wheat growth phase of these experiments as they were fully susceptible to the herbicide or disturbance treatments. The only nutrient applied was N, to rule out any possible effects of *Ornithopus* (a legume) or soil disturbance on N availability to the wheat. The rate applied was 15 mL of 1 M NH_4NO_3 to each pot together with 100 mL of distilled water equivalent to 75 mg N kg^{-1} dry soil. Pots were again watered to 0.17 g g^{-1} by weight. The purpose of watering the pots to weight was to eliminate the possibility of temporary waterlogging and hence the further enhancing of Mn^{2+} ions in the soil solution. Given the sieving of soil in the