



7.4 Arbuscular mycorrhizal symbiosis decreases proline accumulation in plants affected by nematodes

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Content of amino acid proline has been demonstrated to increase in plants under abiotic stress, which is also alleviated by the establishment of arbuscular mycorrhizal fungi (AMF) in roots. This study evaluated the effect of the nematode *Meloidogyne sp.* (N) on proline accumulation in leaves of *Impatiens balsamina*, inoculated or not with the AMF-consortium MTZ-UV (10 g plant⁻¹), and with the application of 150 µg kg⁻¹ of phosphorus (P). A 2x2x2 factorial experiment was established, with 8 treatments and 4 replications. After six weeks of AMF-inoculation, plants were inoculated with *Meloidogyne sp.* and four weeks later plants were harvested. AMF-inoculation significantly ($P \leq 0.01$) reduced proline content, which was higher at non-AMF plants. Neither nematode inoculation nor P-application significantly affected proline content. Plant height, stem diameter, leaf area, number of leaves, and fresh weight were significantly ($P \leq 0.01$) affected by AMF. In addition, significant ($P \leq 0.01$) effects on leaf area and height were observed for P-application, while AMF x P interaction resulted in significant effects on height, and number of leaves. AMF-colonization was not reduced by either nematode or P-application. These results may contribute on understanding physiological mechanisms of AMF-plants involved in enhanced biotic stress tolerance. Further research should be addressed to elucidate the role of proline amino acid in plants affected by the nematode *Meloidogyne sp.*

7.5 In vitro evidences of mycoparasitism of the ectomycorrhizal fungus *Pisolithus tinctorius* by *Hypholoma fasciculare*

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In the northeast of Portugal macrofungi community associated to chestnut tree (*Castanea sativa* Mill.) is rich and diversified. Among fungal species, *Pisolithus tinctorius* and *Hypholoma fasciculare* are common in this habitat. *In vitro* interaction between *P. tinctorius* and *H. fasciculare* was investigated, to ascertain the potential mycoparasitic capabilities of *H. fasciculare* in the growth suppression of ectomycorrhizal *P. tinctorius*. The results show that in co-culture, the growth of *P. tinctorius* was drastically inhibited by *H. fasciculare*. Preliminary studies seem to evidence *P. tinctorius* hyphal injury and stress response in the contact zone between both mycelia. As the chestnut inoculation with these fungi showed higher hyphal adhesion of *H. fasciculare* to the roots in comparison with *P. tinctorius* adhesion, mycoparasitism of *P. tinctorius* by *H. fasciculare* could bring serious consequences to chestnut orchards, even more because the continuous contact of *H. fasciculare* with chestnut roots seems to promote the destruction of vascular root system. In addition, as both fungi exhibit high adhesion capacity to chestnut roots, the involvement of hydrophobins, proteins reported to be involved in the fungus adhesion to other organisms, is being studied by expression analysis in *P. tinctorius* or *H. fasciculare* after being in contact with *C. sativa* roots.

7.6 Impact of the sterol biosynthesis inhibitor (SBI) fungicides on the sterol metabolism of *Glomus intraradices*

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SBI fungicides were used in modern agriculture for control of pests and fungal disease agents. Very little information is available on the effects of SBI fungicides on sterol composition of the non-target arbuscular mycorrhizal (AM) fungi. Up to date, the sterol pathway of AM fungi is not clear. AM fungi are characterized by an absence of ergosterol and by two unusual fungal sterols: the 24-methylcholesterol and the 24-ethylcholesterol. Based on the root-organ cultivation technology, the present study aims to better understand the sterol metabolism of *Glomus intraradices*. Due to the knowledge on different fungicides, on their action sites, and the biosynthetic pathway of other organisms, the objectives of this study are (1) identification of enzymatic target of SBI fungicides, (2) establishment of sterol composition during SBI fungicides application and (3) establishment of the sterol biosynthetic pathway of AM fungi particularly between lanosterol and 24-ethyl or 24-methylsterol. The *in vitro* system using Ri T-DNA transformed carrot roots as the host and were used. Active matter of three SBI fungicides (fenpropimorph, propiconazole, fenhexamid) were tested at various concentrations (0 mg.l⁻¹ (control treatment); 0.02 mg.l⁻¹; 0.2 mg.l⁻¹; 2 mg.l⁻¹; 20 mg.l⁻¹). Sterol analyses were performed on colonized roots and extraradical mycelium. Sterols were analysed by GC and identified by GC-MS.