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ORIGINAL PAPER

Mycorrhizal synthesis between *Pisolithus arhizus* and adult clones of *Arbutus unedo in vitro* and in nursery

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Abstract: Arbutoid mycorrhizae were synthesized between adult selected clones of *Arbutus unedo* L. and *Pisolithus arhizus*. Two micropropagated clones were tested: AL1, *in vitro* and C1 (acclimatized plants) in nursery and later in a field trial. *In vitro*, rooted shoots were transferred to test tubes containing the substrate previously inoculated with mycelium cultured on agar. In the nursery, two inoculation treatments were tested (vegetative inocula or dry sporocarps) and compared to control plants. In the field trial, plants from nursery inoculation treatments were compared and an additional control treatment using seedlings was implemented. Plant height was evaluated 4 months later in the nursery and 20 months later in the field trial. Roots were examined by morphological and histological studies: a) *in vitro* plantlets one month after inoculation and nine months after acclimatization; and b) 20 months after the field trial was established. Arbutoid mycorrhizae were observed *in vitro* one month after inoculation, indicating compatibility between *A. unedo* and *P. arhizus*. These showed the presence of a mantle, Hartig net, and intracellular hyphal complexes confined to the epidermal root cells. Arbutoid mycorrhizae were also observed nine months after acclimatization in inoculated and control plants. In order to confirm the identity of mycorrhizae, molecular techniques were used, in previously inoculated *in vitro* plants, 12 months after acclimatization. *Thelephora* and *Hebeloma* mycorrhizae, two types of highly competitive and widespread mycorrhizae on nurseries were identified. In the nursery, dry sporocarp treatment improved plant height after four months. In a field trial (20 months later), plants growth did not show significant differences. By this time, mycorrhized roots with *Cenococcum geophilum* and other types were identified. These results and their implications on *A. unedo* breeding program are discussed.

Keywords: arbutoid mycorrhizae; Ericaceae; field plants; *Arbutus unedo* or strawberry tree.

Introduction

Arbutus unedo (known as strawberry tree) grows spontaneously in Mediterranean ecosystems. Fruit production represents the main income for farmers. From an ecological perspective, it contributes to biodiversity, helps to stabilize soils, has a strong regeneration capacity following fires, and survives well in harsh environments (Piotto et al. 2001). Because of these characteristics, it has been used in the colonization of marginal lands and to prevent the propagation of forestry fires. For the last five years, we have been involved in a long-term breeding program, in which the main goal is to select and propagate trees to improve the quality of plant material available for orchards, hence enhancing the potential of *A. unedo* as a crop. In close collaboration with farmers, trees have been selected based on fruit production and quality, and protocols for the propagation of these adult trees through axillary shoot proliferation (Gomes and Canhoto 2009; Gomes et al. 2010) and somatic embryogenesis (Gomes et al. 2009; Lopes et al. 2011) were developed.

Mycorrhizae can improve plant adaptation and tolerance to stressful environmental conditions. Species of *Arbutus* (Ericaceae) form mycorrhizae with a broad range of fungal partners (Massicotte et al. 1993). Ultrastructural studies

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