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PISOLITHUS ARHIZUS (SCOP.) RAUSCHERT IMPROVES GROWTH OF ADVENTITIOUS ROOTS AND ACCLIMATIZATION OF *IN VITRO* REGENERATED PLANTLETS OF *PINUS PINEA* L.

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Abstract

Stone pine (*Pinus pinea* L.) is an economically important forest tree in the Mediterranean region and has been the target of breeding and selection through micropropagation mainly for its ecological and ornamental aspects. A crucial step in micropropagation is adventitious rooting of microshoots, which often is highly inefficient in most conifer species including stone pine. Hence, we conducted *in vitro* co-culture of *Pinus pinea* microshoots with the ectomycorrhizal fungus *Pisolithus arhizus* (isolated from natural stands) in order to promote adventitious root growth and plant survival during acclimatization. Significant differences were found in the number of branches, in the number of roots plus branches, in total length of roots, in total length of roots plus branches, in average root length and in the length of the longest root in inoculated plants during *in vitro* rooting compared with non-inoculated plants. The roots of inoculated plants also grew better in vermiculite and during acclimatization in a mixed substrate compared with roots of control plants resulting in the development of vigorous root system. Overall, mycorrhizal inoculation increased the survival rate of the regenerated pine.

Key words: co-culture, *in vitro* mycorrhization, micropropagation, root system.

INTRODUCTION

Stone pine (*Pinus pinea* L.) is an important species widely distributed in the Mediterranean region (Capuana and Giannini 1995). The trees have been exploited for their edible seeds (pine nuts) since prehistoric times and currently, in addition to being cultivated for the seeds, it is also a widespread horticultural tree (Nergiz and Donmez 2004). *P. pinea* has been successfully introduced into North Africa as well as Argentina and South Africa. In other places e.g. California, Scotland, and Southern England it is usually confined to parks and gardens (Fady et al. 2004). In Portugal, there is large-scale production of clonal stone pine genotypes using grafting and *in vitro* organogenesis from mature embryo cotyledons (Alpuim 2000, Carneiro 2002, Zavattieri et al. 2009, Ragonezi et al. 2010a). However, the number of rooted microshoots obtained through organogenesis is low, because similarly to other conifers, stone pine rooting is difficult and genotype dependent (Cuesta et al. 2006, Ragonezi et al. 2010b). Another *in vitro* method of micropropagation is somatic embryogenesis, which in a few pine species is relatively efficient but has limited use in stone pine due to difficulties in somatic embryo maturation and conversion to plants (Carneros et al. 2009).

P. pinea, like other conifers, forms ectomycorrhizas (ECM), which have beneficial impact on plant growth in natural environments (Read 1991) and forest ecosystems (Grove and Le Tacon 1993). Central to the success of these symbioses is the exchange of nutrients between symbionts (Smith and Read 1997) and the extended function of the root system (Smith

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