

New Insights on *Cistus salviifolius* L. Micropropagation

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ABSTRACT

One of the major concerns in the establishment of any mycorrhization program is ensuring the mass production of sterile, consistent and standardized plant material. In the present study, a successful protocol for micropropagation of *Cistus salviifolius* L. was developed. The process was initiated from nodal segments excised from mature *C. salviifolius* plant selected due to its mycorrhizal capacities. Murashige and Skoog basal medium supplement with gibberellic acid (0.5 mg/L) and of 6-Benzylaminopurine (0.5 mg/L) was the best medium for proliferation purposes and successful rooting was achieved with the same basal medium supplemented with Indole-3-butyric acid (0.5 mg/L). The proposed methodology represent a novelty because it allowed the rapid multiplication of *C. salviifolius* starting from mature explants, here reported for the first time, using lower plant growth regulators concentrations than the previously reported for this particular *Cistus* species

INTRODUCTION

The genus *Cistus* L. (*Cistaceae*) is one of the most characteristic genera of the Mediterranean flora ^[1]. It encompasses a group of about 20 perennial shrub species, distributed throughout the Mediterranean region and Canary Islands, all sharing the same distinctive feature, a combination of diverse hair types on the leaf, stem, and calyx ^[2,3]. *Cistus* species exhibit a range of specific adaptations as well to Mediterranean environments, such as, fire-dependent seed germination, insect-dependent pollination, flower-dependent reproduction and spring-dependent phenology ^[4].

Cistus species are involved in many ecological processes taking place in Mediterranean ecosystems ^[5]. Furthermore, they support a vast and rich mycobiota, constituting reservoirs for mycorrhizal fungal inoculum in the absence of host trees ^[6]. In total, more than 200 fungal species, belonging to 40 genera, have been reported to be associated with *Cistus*. Among which, several edible hypogeous *Ascomycota*, mainly included in *Tuber* and *Terfezia* genera, and commonly known as truffles ^[7]. Truffles are highly sought-after and some species command extraordinary prices in local markets, however, due to their ectomycorrhizal nature, truffles must be cultivated in orchards with their plant hosts ^[8]. As proposed by Giovannetti and Fontana, the wide variety of *Cistus* (and other *Cistaceae*) environmental and ecological requirements makes them ideal candidates to increase the range of habitats where truffles can be grown ^[9]. Thereby, inoculating these *Cistaceae* with truffle inoculum and planting them in a primary stage of truffle forest repopulation, has become an extremely interesting new use for *Cistus* plants and one with great economic importance and potential for forestry purposes ^[10].

Cistus salviifolius L. a low subshrub up to 1 m tall, with ovate to rounded leaves and white flowers is the most widely spread species of the genus around the Mediterranean basin ^[3]. It can occur in sandy soils over a wide range of habitats and has been regularly reported as a plant host for various *Terfezia* species, which makes it one of the best choices for planned *Terfezia* cultivation over a wide range of habitats ^[7,11,12].