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mycorrhizas: A link with other types
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Superoxide Dismutase Activity in Mycorrhizal and Non-Mycorrhizal Pea (*Pisum sativum* L.) Plants

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Summary.- Among the changes in the protein expression as a consequence of the arbuscular mycorrhizal (AM) symbiosis, only one case related to changes in superoxide dismutase (SOD; EC 1.15.1.1) activity has been reported in the red clover-*Glomus mosseae* symbiosis. In this work, the SOD isoenzymatic pattern of the symbiotic system formed by a leguminous plant, *Pisum sativum*, and the AM fungus *Glomus mosseae*, was studied in comparison with non-mycorrhizal roots. A higher SOD activity was detected in mycorrhizal pea roots, although both mycorrhizal and non-mycorrhizal roots showed the same isoenzymatic pattern for SODs: two Mn-SODs (I and II) and two Cu,Zn-SODs (I and II) were detected, Cu,Zn-SOD I being the most abundant isozyme in both types of roots. A similar pattern of SOD isozymes (Mn-SODs I and II, and Cu,Zn-SODs I and II) was also found in nodules of mycorrhizal and non-mycorrhizal pea roots. However, in nodules Mn-SOD II was the main isozyme. A partial purification of the different isozymes could be achieved by DEAE-cellulose chromatography.

Keywords: arbuscular mycorrhizal fungi, isozymes, nodules, *Pisum sativum*, superoxide dismutase

Introduction

Increasing attention is being given to the study of the biochemical processes involved in mycorrhization. The first difficulty of this research is to know the role that the proteins differentially expressed play in the symbiosis. One possibility is the study of some enzymatic activities, as have been reported (9, 11, 12). New isozymes of acid phosphatase (11) are induced by arbuscular mycorrhization. In a recent study carried out in our laboratory, an extra band with superoxide dismutase (SOD) activity was detected in mycorrhizal root extracts after isoelectric focusing (1), and it was also found that one manganese-containing superoxide dismutase and one copper,zinc-containing superoxide dismutase were induced in the red clover-*Glomus mosseae* symbiosis (12).

In this paper, the symbiotic system *Pisum sativum*-*Glomus mosseae* was studied, and data concerning protein patterns and SOD activity of non-mycorrhizal and mycorrhizal root extracts are given.

Materials and Methods

Pregerminated *Pisum sativum* L. (cv. Petit Provençal Bajo) seedlings were grown in a sterilized sand/vermiculite (1/1, v/v) mixture, and were either left uninoculated or inoculated with soil-inoculum (10%) of *Glomus mosseae*. Filtered leachates of the inoculum were applied to non inoculated plants to compensate for the free-living microflora associated with the mycorrhizal inoculum. Plants were grown in a growth chamber under controlled conditions. Due to the nature of the filtrates used in our growth conditions, both mycorrhizal and non-mycorrhizal plants were grown in the presence of natural *Rhizobium* strains. Plants from each treatment (uninoculated and inoculated) were taken after 18, 21, 23, 25, 28 and 30 days after starting the experiment. Fresh weight of shoots and roots, as well as the percentage of root colonization (%I) in mycorrhizal roots, were determined.

Root samples were extracted under liquid nitrogen with 0.1 M K-phosphate buffer, pH 7.0, containing 0.1% Triton X-100 and 10 mM ascorbic acid. The suspension was filtered, and centrifuged at 15,000 rpm in a microcentrifuge for 5 min. The supernatant was chromatographed through a DEAE-cellulose column, using 10 mM K-phosphate buffer, pH 7.9, as equilibrating buffer. After washing the column with the buffer, a double elution with 0.2 M NaCl, and then with 0.7 M NaCl in the same buffer was performed.

Total superoxide dismutase activity was determined by following the reduction of ferricytochrome *c* by superoxide radicals generated by a xanthine/xanthine oxidase system (10). SOD isozymes were separated by nondenaturing PAGE on 10% acrylamide slab minigels, and detected by the method of the photochemical reduction of Nitroblue Tetrazolium (2). The different types of SOD were identified by performing the activity stain in gels previously incubated for 30 min at 25 °C, in 50 mM K-phosphate buffer, pH 7.8, containing either