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Xyloglucan-specific Endoglucanase Activities in Onion Plants Colonized by the Arbuscular Mycorrhizal Fungus *Glomus mosseae*

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Summary.- We found that the extract from the external mycelium of *Glomus mosseae* had xyloglucan-specific endoglucanase activity. This activity was detected using nasturtium seed xyloglucan as substrate (xyloglucanase). We studied the possible involvement of the hydrolytic enzyme endoxyloglucanase during the process of penetration and development of the arbuscular mycorrhizal fungus *G. mosseae* in roots of onion (*Allium cepa* cv. Babosa).

Keywords: Glomus mosseae, *hemicellulase, hydrolytic enzymes, arbuscular mycorrhiza, xyloglucan-specific endoglucanase*

Introduction

The establishment of arbuscular mycorrhizal (AM) symbiosis requires penetration of the host cell by the (AM) fungi. There is evidence that hydrolytic enzymes (cellulases, hemicellulases and pectinases) are involved in the colonization of the root by the fungi. The observation that AM fungi penetrate the plant cell wall at the site of contact (1), and the fact that spore extracts of *Glomus mosseae* contain pectinolytic and cellulolytic enzymes (5, 6) indicate that these hydrolytic enzymes may be involved in the colonization process.

Xyloglucans are the hemicellulosic polysaccharides that account for 20 to 25% of the dry mass of the primary cell walls in dicotyledons. They consist of a backbone of β-(1-4) linked D-glucosyl residues and are hydrogen bonded to cellulose (9). Xyloglucan molecules may act as "strings", tethering adjacent microfibrils and thereby contributing to the coherency of the cell wall. If xyloglucan serves such a key "tethering" role in the cell wall, enzyme cleavage of the xyloglucan backbone could loosen the cell wall and facilitate turgor-driven cell expansion (3, 4). To date, no studies on the participation of hemicellulase enzymes in the process of AM infection have been published.

The aim of this work was to study the presence of the hemicellulose xyloglucan-specific endoglucanase (xyloglucanase) in external mycelium of *Glomus mosseae*, and the potential role of this enzyme in the process of colonization of onion roots.

Materials and Methods

Plants were grown in 300 ml pots containing soil collected from the province of Granada, Spain. The soil was a calcexerollic xerochrept type, pH 7.6 (7). It was steam-sterilized and mixed with sterilized sand (1/3, v/v). Onion (*Allium cepa*, cv. Babosa) was used as test plant. Seeds were sown in moistened sand and after two weeks seedlings were transplanted to the pots and grown under greenhouse conditions. Natural light was supplemented by Silvania incandescent and cool-white lamps providing 400 nmol $m^{-2}s^{-1}$ (400-700 nm), with a 16-8 h light-dark cycle at 25-19° C and 50% relative humidity. Plants were watered from below using a capillary system, and fed with a nutrient solution (11) lacking phosphate for AM-inoculated plants. The AM inoculum consisted of 5 g of rhizosphere soil from an onion plant pot culture of a *G. mosseae* isolate which contained spores (15 sporocarps per g with 1-5 spores per sporocarp), mycelium and colonized root fragments. Uninoculated plants were given filtered leachings from the inoculum soil (Whatman No. 1 filter paper). The filtrate contained common soil microorganisms, but no propagules of *G. mosseae*.

Plants were harvested after 7, 15, 30, 50 and 80 days. The root system was washed and rinsed with sterilized distilled water, and aliquots of the root system from each of the five replicate groups of pots were cleared and stained (11), and examined under a compound microscope.