Symbiosis, 26 (1999) 55–63
Balaban, Philadelphia/Rehovot

Xyloglucan Endotransglycosylase Activities in Onion Roots Colonized by the Arbuscular Mycorrhizal Fungus *Glomus mosseae*

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Received February 8, 1998; Accepted April 27, 1998

Abstract

We studied the production of xyloglucan endotransglycosylase (XET; EC 2.4.1.-) activity during the process of penetration and development of the arbuscular mycorrhizal (AM) fungus *Glomus mosseae* in roots of onion (*Allium cepa*, cv. Babosa). Viscometry assays did not yield any detectable transglycosylation products; however, a radiochemical assay showed that in both mycorrhizal and nonmycorrhizal plants XET activity decreased as the root grew. Xyloglucan endotransglycosylase activity was higher in mycorrhizal plants than in non-mycorrhizal plants. The possible role of XET associated with wall loosening in the colonization of the plant root by AM fungi is discussed.

Keywords: *Allium cepa*, arbuscular mycorrhiza, *Glomus mosseae*, xyloglucan endotransglycosylase

1. Introduction

Arbuscular mycorrhizal (AM) symbioses are widespread throughout the plant kingdom. They may benefit the host plant primarily by increasing the capability of the root system to absorb and translocate phosphorous through an

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extensive network of external hyphae (Hayman, 1983). The colonization of roots of many plant species including *Allium* spp. by AM fungi involves the formation of highly branched intercellular hyphae and intracellular arbuscules and vesicles scattered throughout the root (Bonfante-Fasolo, 1984). The establishment of an intracellular symbiosis between the fungus and plant roots requires penetration of the host cell wall by the fungus. Cell-wall hydrolyzing enzymes such as cellulases, hemicellulases and pectinases may be involved in this process (García-Romera et al., 1990).

Host plasmalemma and AM fungal walls are always separated by interfacial material of host origin. The different types of interface depending on whether the fungus does or does not penetrate the host cell, are composed of molecules common to the primary cell wall. The presence of these molecules indicates that the interface, retains the enzymatic machinery involved in synthesis and secretion of cell wall material. The presence of the fungus in the plant cell seems therefore to affect only the assembly and not the expression of molecules usually present at the host cell surface (Bonfante, 1994).

The universaly of xyloglucan occurrence in the primary cell walls of higher plants and the notable decrease in xyloglucan content during plant growth indicate its importance in cell wall metabolism (Reid, 1985). Wall loosening, and thus growth, may be facilitated by the cleavage of xyloglucan chains (Labavitch and Ray, 1974). Endotransglycosylation could provide such a mechanism, and an enzyme, xyloglucan endotransglycosylase (XET), capable of catalyzing xyloglucan endotransglycosylation, has been described recently (MacDougall and Fry, 1990; Smith and Fry, 1991; Farka et al., 1992; Fry et al., 1992b; Nishitani and Tominaga, 1992). XET cuts a xyloglucan chain and then conserves the energy of the cleaved glycosidic bond in the formation of a chemically identical bond, restoring the strength of the cell (Nishitani and Tominaga, 1992). XET is thought to contribute to cell elongation through wall loosening.

In this study, we have examined whether XET is associated with the colonization of plant roots by AM fungi, a process characterized by alterations in the cell wall

2. Materials and Methods

Plant materials

Plants were grown in 300 ml pots in soil collected from the province of Granada, Spain. The soil, a Calcexerollic Xerochrept type, pH 7.6 (for details see García-Romera and Ocampo, 1988) was steam-sterilized and mixed with sterilized sand (2:3, vol:vol).

Onion (*Allium cepa* L., cv. Babosa) was used as the test plant. Seeds were sown in moistened sand. After two weeks, seedlings were transplanted to the pots and grown under greenhouse conditions. Sun light was supplemented by Sylvania incandescent and cool-white lamps, 400 nmol m^{-2} s^{-1}, 400–700 nm; with a 16-8 light-dark cycle at 25–29°C and 50% relative humidity. Plants were watered regularly and fed with a nutrient solution (Hewitt, 1952) which lacked phosphate for the AM plants.

The inoculum consisted of 5 g of rhizosphere soil from alfalfa-plant pot cultures of an isolate of *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe, which contained spores, mycelium and colonized root fragments. Uninoculated plants were given a filtered leachate from the inoculum soil. The filtrate contained common soil microorganisms, but no propagules of *G. mosseae*.

The external mycelium was isolated from roots of 50-day-old onion plants colonized with *G. mosseae*. The roots were first washed and rinsed gently with sterilized water, and then the external mycelium was collected with forceps under a dissecting microscope (García-Garrido et al., 1992).

Plants were harvested after 7, 15, 30, 50 and 80 d. The root system was washed and rinsed with sterilized distilled water, and aliquots of the root system from each group of pots were cleared and stained (Phillips and Hayman, 1970). The percentage of total root length that was colonized by AM fungi was measured by the grid-line intersect method (Giovannetti and Mosse, 1980).

Extraction of soluble XET activity

Roots (5 g fresh weight) were pulverized in a mortar under liquid nitrogen. The resulting powder was homogenized in 10 ml of 25 mM MES (Na^+) buffer, pH 6, 10 mM $CaCl_2$, 10 mM ascorbate. The homogenate was centrifuged (2000 g, 10 min) and the supernatant was assayed for XET.

External mycelia were extracted as described above. The resulting powder was suspended (30 mg ml^{-1}) in the same extractant solution as for roots. The suspension was briefly sonicated (for 1 min, 5 times at 80 W), centrifuged at 20,000 g for 15 min. The pellet was resuspended and sonicated again, and washed by centrifugation with the same buffer three times. The supernatant was concentrated by ultrafiltration using PM-10 membranes (Amicon), and used as a crude enzyme extract. Protein content of samples was determined by the method of Bradford (1976) using the Bio-Rad kit with BSA as the standard.

Enzyme assay

Xyloglucan endotransglycosylase activity was determined by a method

similar to that of Fry et al. (1992b). Xyloglucan was isolated from nasturtium (*Tropaeolum majus* L.) seeds by the method of McDougall and Fry (1989). The substrate solution contained 2 mg ml^{-1} of nasturtium xyloglucan, 50 mM MES (Na^+), pH 6, and 75 KBq/ml [^{3}H]XXXGol (formerly XG7-ol, for nomenclature, see Fry et al., 1993). Reaction mixtures consisted of 20 µl of substrate solution and 20 µl of enzyme extract. Time-course assays were done at 25°C; the reactions were stopped by the addition of 100 µl of 20% (wt:vol) formic acid. The products were dried on a 5 × 5 cm square of Whatman 3MM chromatography paper, which was then washed for 1 h in running tap water to remove the unreacted [^{3}H]XXXGol, and redried at 60°C. The squares were placed, with the loaded side outermost, in 22 ml scintillation vials, soaked with 2 ml of scintillant (Ecoscint H) and assayed for paper-bound [^{3}H]xyloglucan by scintillation counting (efficiency was approximately 44%). The results were expressed as cpm incorporated into polymeric xyloglucan per hour. The data were plotted as the means of three replicate assays.

Viscometric assay for xyloglucan depolymerization

A solution of nasturtium seed xyloglucan (500 µl, 8 g l^{-1}) was mixed with 126 µl of enzyme extract and incubated at 25°C. After 30 min, 400 ml of the solution was transferred into a new tube containing the appropriate amount of dry oligosaccharide to give the final concentration indicated (25 µM). At intervals, the solution was sucked into a 0.2 ml pipette and the efflux time was determined at 25°C (Lorences and Fry, 1993).

3. Results

Microscopic observations of stained roots showed no AM fungi in control plants, and AM structures in inoculated plants. Percentage AM root colonization of onion from day 7 after transplanting showed the usual logarithmic growth pattern until 80 d (Fig. 1).

Viscometric assay

To test for possible interfering enzyme activities in our enzyme preparation, we incubated [^{3}H]XXXGol in the presence of the onion root extracts under the conditions used for viscometric assays. Chromatography of the radioactive products on paper showed no detectable α-D-xylosidase activity (data not shown), which might have interfered with the assay by removing the essential xylose residue (Lorences and Fry, 1993). The 15-d onion root extracts from AM and nonAM plants did not show any XET activity (assayed

viscometrically, Fig. 2). Similar data were obtained with the other onion root extracts (day 7, 30, 50, 80; data not shown). The viscometric assay with xyloglucan as the substrate showed that high xyloglucanase activity was present in the enzyme extracts used (Fig. 2).

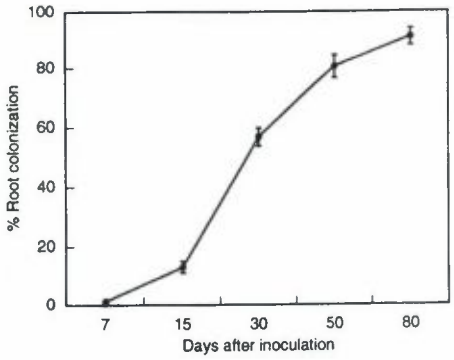

Figure 1. Percentage of root length colonized in onion plants inoculated with *G. mosseae*. Data points represent the means of 3 replicates ±SE.

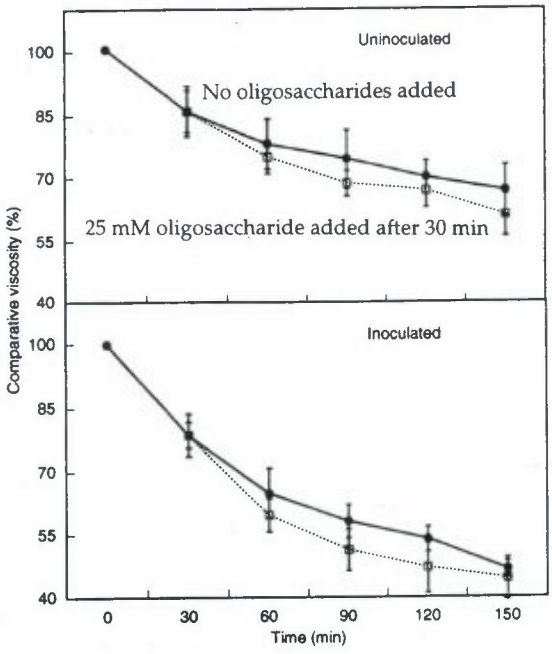

Figure 2. Kinetics of the loss of viscosity of xyloglucan in the presence of 15-day-old extract from onion roots, uninoculated and inoculated with *G. mosseae*. Data prints represent the means of 3 replicates ±SE.

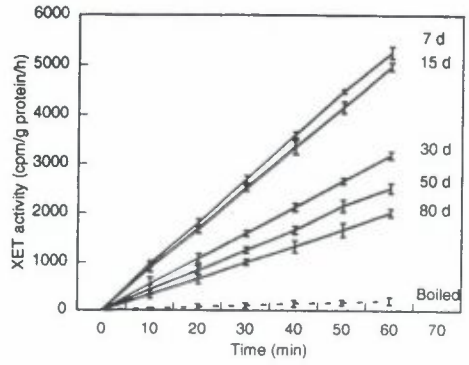

Fig. 3.

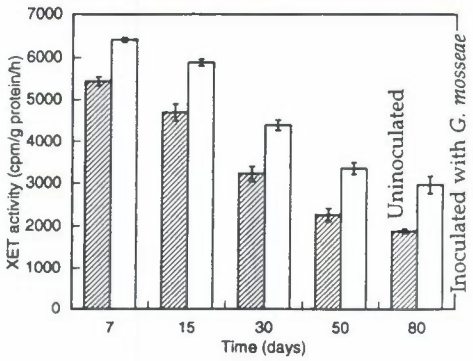

Fig. 4.

Figure 3. Time-course assays of XET activity in extracts from uninoculated onion roots at different stages of growth. The broken line represents uninoculated onion root extract boiled for 5 min before assay. Data prints represent the means of 3 replicates ±SE.

Figure 4. XET activity in onion roots. Standard errors of means are given. Data prints represent the means of 3 replicates ±SE.

Radiochemical assay

The growing onion roots had detectable XET activity (Fig. 3) at all times tested. A time-course assay demonstrated linear kinetics over 1 h in onion plants of different ages; routine assays were conducted for 1 h. No enzyme activity was detected after boiling the root extracts (Fig. 3).

Xyloglucan endotransglycosylase activities in AM and nonAM plants decreased with time (Fig. 4). Mycorrhizal onion plants showed more XET activity than nonmycorrhizal plants throughout the experiment. No detectable XET activity was found in the external mycelium of *G. mosseae*.

4. Discussion

Xyloglucan endotransglycosylase activity has been found in extracts from the growing portions of monocots and dicots, and in cell suspensions (Fry et al., 1992a; Nishitani and Tominaga, 1992; Pritchard et al., 1993). This enzyme is thought to be important in the mechanism of plant cell elongation. This experiment was designed to explore the idea that XET is associated with wall loosening in the context of mycorrhizal association, a process characterized by alterations in the cell wall.

Xyloglucan is subject *in vivo* to both endohydrolysis (by xyloglucanase), detected by the viscometric assay, and endotransglycosylation (by XET), detected by viscometric or radioactivity assays (Fry et al., 1992a). Although we might expect to find transglycosylation in onion roots, since this may be an important process in cell wall modification, XET was not detectable by viscometric assay. The lack of XET activity suggests that contaminating hydrolases such as xyloglucanase interfered with the XET assay. Our results demonstrated that our enzyme extracts were not appropriate for a study of the effects of xyloglucan oligosaccharides on xyloglucan depolymerization.

Xyloglucan endotransglycosylase activity can also be monitored by measuring the incorporation of radioactive oligosaccharide substrates (Smith and Fry, 1991) into higher molecular weight xyloglucan. Data obtained with this technique showed that XET was present in onion roots, and its activity decreased rapidly as the root grew due to the decrease of the elongation process. This pattern is the reverse of the infection process (Figs. 1 and 3). In addition, AM infection is not related with an elongation process, since colonization occurs in cells which are already differentiated and elongated. All these aspects suggest that XET activity is not closely related to the penetration of AM fungi into cortical root cells. However, our results show that XET activity was higher in AM than nonAM plants, suggesting that XET may be involved in the process of root colonization by AM fungi. The lack of XET activity in the AM mycelium suggests that this activity may be produced either by the fungus inside the root or by the root itself after induction by the fungus. Colonization of roots by arbuscular mycorrhizal fungi can result in significant alterations of the plant root system morphology (Berta et al., 1990; Atkinson et al., 1991; Hooker et al., 1992). The combined effects of increases in branching and lengths of individual roots due to colonization by AM are not entirely due to improved host plant nutrition and are likely to involve AM fungus interaction with plant cell cycles (Hooker and Atkinson, 1996). The change in the root system could require an intense XET activity, the induction of some XET genes in AM plants support this idea (Harrison et al., 1997).

The methods used for XET detection are based on those previously shown to be highly effective for the measurement and characterization of XET activity (Fry et al., 1992b). However, the presence of an enzyme which has the ability to degrade xyloglucan chains, and possibly the low levels of XET make it difficult with these techniques to detect the different XET activities in the process of host-wall degradation-restoration during the AM colonization process. Further studies with purified XET will be aimed at developing more sensitive techniques (EM localization using immunogold complexes with antisera to XET) to more fully elucidate the role of the transglycosylate enzyme during AM colonization.

Acknowledgments

We wish to thank Dr. S.C. Fry for the kind gift of [^{3}H]XXXGol, Esther Lorences for the oligosaccharides, Ms. Joyce Aitken for preparing the nasturtium xyloglucan, and Karen Shashok for grammatical correction of the text.

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