PRODUCTION OF CELL WALL ENZYMES IN PEPPER SEEDLINGS, INOCULATED WITH ARBUSCULAR MYCORRHIZA *GLOMUS ETUNICATUM*

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ABSTRACT

Pepper seedlings inoculated with arbuscular mycorrhizal AM fungus, Glomus etunicatum, *produced cellulase, polygalacturonase and pectin methylestrase enzymes. The activities of the enzymes increased as the pepper seedlings matured in age, showing that the activity of the enzymes in the seedlings was age mediated. The pectinases showed higher activity than the cellulase enzyme which may indicate establishment of the mycorrhizal in the host. The significance of this finding is that there is an initial infectivity factor in (AM) fungi interactions with plant just like in other host-parasite interaction.*

INTRODUCTION

Natural infection of host plants by AM-fungi occurs through the germination of fungal spores present in the soil into a germ tube. The tube then develops into a hypha that penetrates the epidermal surface of the plant roots through the appressorium, usually between or through sub-epidermal cells where hyphal coils may be formed (Sander & Sheikh 1983). It is generally accepted that enzymes hydrolysing the main components of the cell wall of plants (cellulose and pectin) allow disintegration of living and dead tissues (Wood 1960, Bateman & Miller 1966, Goodman *et al.* 1986), thus enabling the organisms to enter the root.

The ability to synthesize cellulolytic and pectolytic enzymes is wide spread among plant pathogenic microbes (Ikotun 1984, Perez-Artes & Terra 1989, Oladiran & Oluma 1990) and saprophytic microorganisms (Kuster 1979,, Strzelezyk & Szpotanski 1989). These enzymes may also be of importance in mycorrhiza formation. Some of the ectomycorrhiza forming fungi have been found to produce such enzymes (Lindeberg & Lindeberg 1977, Dahm & Strzelczyk 1995). However, authors have not been unanimous on cellulose

and pectin degradation in mycorrhizal fungi. The cell wall degrading enzyme produced by pathogens are subject to catabolic repression brought about by the accumulations of sugar in cells of plants or organisms synthesizing the enzyme (Arinze *et al.* 1976). Several mycorrhiza fungi have been shown to express proteolytic activity which appears to be important for growth of the mycelia and mycorrhizal plant (Leake & Read 1989, Maijala *et al.* 1991, Zhu *et al.* 1994). Mycorrhizal formation in plants begins with spore germination, followed by colonisation and penetration into host root tissue. The biological factors affecting the formation of mycorrhizal association can not be excluded. The present study was initiated to determine the production of hydrolytic enzymes in the interaction of arbuscular mycrorriza fungi and the inoculated plant. To the author's best knowledge this is the first time such an interaction factor will have been investigated in Nigeria.

METHODS

Propagation of mycorrhiza

The mycorrhizal inoculum, which is the mycorrhizal spores in both the soil and the root fragments (soil containing mycorrhiza spores and root fragments) was obtained from the soil biology unit of the Department of Botany and Microbiology, University of Ibadan. *Glomus etunicatum* was chosen and propagated as per procedure of Powell (1982).

Inoculation of pepper seedlings

The pure inoculum of propagated AM consisting of soil, spores and root fragments was used in inoculating pepper seedlings. The pepper seedlings were inoculated with the mycorrhizal *Glomus etunicatum* at seedling ages 2,4,6,8,and 10. Mycorrhizal inoculation was affected by placing 30 grams of AM inoculum directly below each seedling which was then drenched and covered back with sterile soil in the plastic pot.

Enzyme extraction

Pepper seedlings that were inoculated at different ages were harvested 96 hours later. Their roots and stems were excised with sterile blade and taken into the laboratory where they were washed clean of soil debris using sterile distilled water, before grinding in mortar for enzyme extraction.

Cellulase activity

Assay procedure used to determine cellulase activity was that according to Somogyi (1952) and as per the later modifications (Hurst *et al.* 1977, Hakansson *et al.* 1979, Ooshima *et al.* 1986). This is a photometric method using copper reagents and arsenomolybdate reagent. One hundred milligrams of carboxyl methylcellulose (CMC) (Sigma Chemical CO) was dissolved in 10 ml of 0.1 M Sodium Acetate (NaAC) buffer at pH 4.8. One half milliliter of CMC was measured into the test tube together with 0.1 ml of the enzyme extract. Both were mixed and then incubated at 40°C for 30 minutes. The

blank was also prepared by measuring 0.5 ml of CMC and 0.1 ml of sterile distilled water into another test tube and was also incubated at 40°C for 30 minutes.

After 30 minutes incubation, 0.6 ml of reaction mixture (i.e. enzyme extract and the blank) was taken through Somogyi – Nelson's procedure for determining cellulase activity. This was done by adding 0.4 ml of sterile distilled water to each of the reaction mixture; 1.0 ml of combined reagent, (i.e. reagent A; which consisted of the following: 2.5 g Na_2CO_3 (anhydrous) 2.0 g $NaHCO_3$, 2.5 g potassium sodium tartarate, and 2.5 g sodium sulphate plus reagent B, which consisted of 15% copper sulphate solution, and two drops of concentrated sulphuric acid were added and then heated at 100°C for 20 minutes. After heating, 1.0 ml of Arsenomolybdate reagent and 7.0 ml of sterile distilled water were measured and added to the reaction mixture. The optical density was then taken at 540 nm on cecil (CE) 595 Double Beam Digital U.V. spectrophotometer.

Pectin Methylesterase (PME) activity

The assay method used was a modification after Winstead and Walker (1954). The reaction mixture was as follows: 1.2 g of pectin was weighed and dissolved in 100 ml of acetate buffer (pH 4.5), 10 ml of the 1.2% pectin solution was dispensed into three replicate test tubes along with 1 ml of 0.5 M NaCl solution and 1.5 ml of the extract. These were mixed together and incubated at 30°C for 3 hours. The resultant solution was filtered against 0.1 M NaOH using phenolphthalein indicator. The unit of PME activity was calculated.

Polygalacturonase (PG) activity

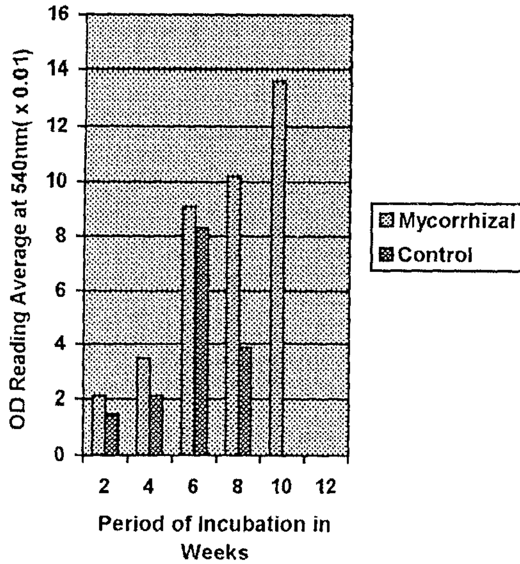
Polygalacturonase activity was assayed using the method of Winstead and Walker (1954). The reaction mixture consisted of 1ml of 0.55% pectin in citrate phosphate buffer (pH 5.0) and 1 ml of pepper enzyme extracts in three replicate test tubes. This reaction mixture was incubated at 30°C for 3 hours. At the end of the incubation period, the mixture was boiled for 15 min in a water bath and then cooled under running water. The optical density (OD) showing the level of activity of PG was read at 575 nm on a Cecil (CE) 595 Double beam digital U.V spectrophotometer. A unit of PG activity is taken as a change of 1.0 mn O.D at 575 nm.

RESULTS AND DISCUSSION

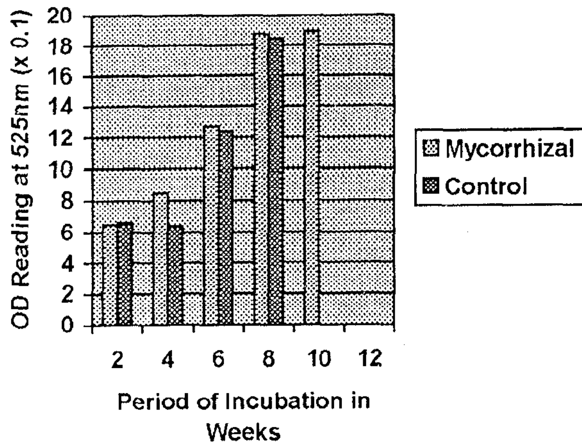
There were increases in the activities of cell wall enzymes in pepper seedlings inoculated with arbuscular mycorrhiza fungus (Fig. 1). Cellulase and polygalacturonase activities of the pepper plant increased as the age of the seedling increased while pectin methyl esterase enzyme activity decreased (Fig. 1b and c). The increase in the pectinases in pepper plant was significant, in that the pectinase enzymes caused soft rot of plant tissue. This might allow

the mycorrhiza hyphae to penetrate more into the cell-lumen of the pepper plant for further establishment. The ANOVA test showed that the level of activity among the treatment differed significantly at the 0.01 level of confidence.

Cellulase Activity



Polygalaturonate Enzyme Activity at 525nm



Pectin Methyestrase Enzyme Activity

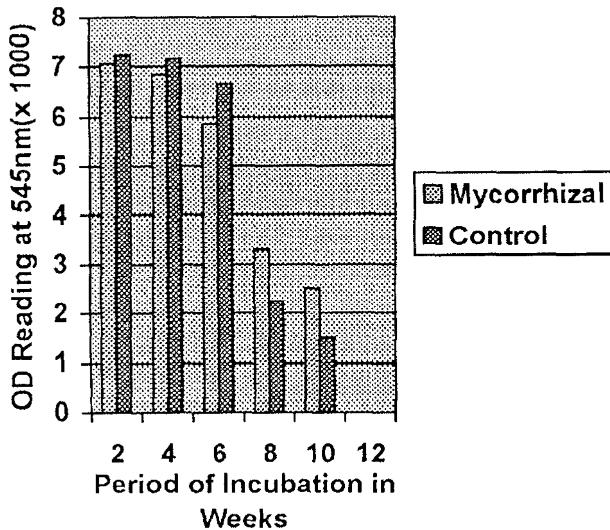


Fig. 1(a-c): Cell wall enzymes activities (OD reading averages at 540, 525 and 545) in mycorrhiza inoculated pepper seedlings

Thus the inoculation of pepper seedlings with *Glomus etunicatum,* an AM fungus, resulted in the production of cell-wall degrading enzymes. This suggests that at least some mycorrhiza are capable of producing such an enzyme. This may mean that the natural infection of host plant by AM fungi occurs through the germination of spores present in the soil. That the penetration of the epidermal surface of the roots by the germ tube

(appressorium) formed by the spores may be caused by enzymatic factors. Earlier opinion has been that the mycorrhiza fungi are not able to enter the roots because of lack of cell-wall degrading enzymes (Sanders & Sheikh 1983).

Further evidence for the production of cell wall degrading enzymes by arbuscular mycorrhizal fungi, was the reduction in plant growth and attainment of chlorotic symptoms, at the early stage of mycorrhiza infection. Chlorosis was a physiological (rather than pathological) effect because this symptom disappeared as soon as the mycorrhizal established in the inoculated plant. On the other hand it could be argued that since such physiological chlorosis was caused by secretion of extracellular enzymes from pathogenic fungi; at onset of infection, it is principally pathological.

As plant matures, the epidermal cells (layer) thickens making it difficult for pathogens to penetrate and this may cause increase in the secretion of extra cellular enzymes, the chemical weapon which the pathogen would have used in invading the host cell. The host (plant) gains from the symbiosis are usually influenced by a number of factors like mycorrhizal compatibility with host, the physiological shock which may lead to chlorotic symptoms and the improved nutrition.

The increase in the level of activity of the extra-cellular enzymes found for all ages could be caused by its response to developmental stages of the plant. It can therefore also be a form of in built resistance with age. Furthermore, the increase in the extra-cellular enzyme activities could be attributed to a shift in the balance of establishment of *Glomus etunicatum* mycorrhiza, (an obligate symbiont fungus) infection into plants (Chou & Schlumitthener 1974, Osonubi e*t al.* 1990). This can also be a form of source-link relationship as observed by Martins (1992) and Newman *et al.* (1992).

The present study suggests that mycorrhizal fungi, especially arbuscular mycorrhiza, may also produce cell wall degrading enzymes in their symbiotic relationship with plant hosts as has been found in ectomycorrhizal and pathogenic fungi.

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