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Ultra-Structural Cellular Changes in chili pepper Roots Induced by Arbuscular Mycorrhizal Fungi Colonization

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ABSTRACT

The aim of this study was to investigate the efficiency of simultaneous and spatial inoculation Actinomycetes and Mycorrhizal fungi in the control of Fusarium wilt in pepper, and their effects on the cell structures of the infected plants using Scanning Electron Microscope. SEM observations showed that chilli root cell walls can colonize intensively by *G. mosseae*. The presence of *G. mosseae* structures in an enormous amount was attributed to a cell wall lignification. Net of vehicles in addition to Mature spores of *G. mossea*e and arbuscular were shown attached to the root cell in Scanning Electron Microscope observation. A Large number of small vacuoles observed as a response to the heavy colonization by *G. mosseae.* The colonization by *G. mossea*e occurred during the hyphae structure between root epidermal cells and the vast number of nuclei. The nuclei are observed in colonized cells and new entry point in the cell wall. Endophytic *G. mosseae* penetrates the root and grow expansively between and within living cortical cells and affects a lot of aspects of root metabolism.

Keywords: Arbuscular mycorrhizal fungi, Scanning electron microscopy. Cell wall, Arbuscular.

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INTRODUCTION

Chilli (*Capsicum annum* L.) is fruit-vegetable that usually found in multiethnic of Malaysian's daily food menu. It is extremely popular for the enormous content of vitamin C and total soluble phenolics elevated than another vegetables commonly recognized as a source of this substance (Marinova *et al*., 2005; Anil Kumar *et al*., 2009). The major disease of chilli crop is *Fusarium* wilt caused by the soil-borne pathogen *Fusarium oxysporum* (W. C. Snyder & H. N. Hans, 1940). It is a common disease in *Solanaceae*, e.g. in chilli, tomato, eggplant, and potato. Once the field is infested, the pathogen may survive in the soil for many years. The pathogen infects young root, developing, growing and spreading in root and stem the vessel, inhibiting nutrient transport and water (Miller *et al*., 1986). Various fungicides have been applied against Fusarium wilt to control the disease in infected areas. While fungicides can play a significant role in the disease control, they can adversely affect other useful soil microorganisms as well as contaminate the environment (Parker *et al*., 1985). So, biocontrol methods, based on the use of beneficial microbe isolated from suppressive soils, represent an alternative for protection the plants against Fusarium wilts (Alabouvette *et al*., 1993).

The arbuscular mycorrhiza (AM), a symbiosis formed between land plants and arbuscular mycorrhizal fungi (AMF), is widespread. This is indicated by the percentage of land plants forming this symbiosis, which is about 70-90% (Qiu, 2006). Mycorrhizae are ecological and economical important as they can improve pathogen resistance (Vigo *et al*., 2000; de la

Pena *et al*., 2006) as well as biomass production (Smith *et al*., 2008) of the host plant. In addition, mycorrhizae mitigate different kinds of plant stresses such as drought (Michelson & Rosendahl, 1990; Auge *et al*., 2001; Aroca *et al*., 2007), or heavy metal toxicity (Hildebrandt *et al*., 1999) and protect plants against root herbivores (Gange, 2001). Specific bacteria together with mycorrhizae fungi may create a more indirect synergism that supports plant growth (Barea, 1997), including with nutrient acquisition (Barea *et al* ., 2002), inhibition of plant pathogenic fungi (Budi *et al* ., 1999), and improvement of root branching (Gamalero *et al* ., 2004).

MATERIALS AND METHODS

Growth conditions and Biological Materials:

This study was conducted at the University Putra Malaysia. Institute of Bioscience (IBS) Healthy and mature spores was isolated and collected from the pot culture. 100 spores for 100 gram dry soil were added to the containers (20 X 20 cm) and mixed well. A commercially and certified chilli cultivar is used. The seeds were surface sterilized with 90% ethyl alcohol for 10 sec and washed with sterile distilled water. For seeds were planted directly into the pot. Two weeks later, the seedlings were thinned to 1 seedling/pot. The pots were kept under greenhouse conditions (25-30C°) for two months to get greatly colonized roots for the study.

Colonization assessments:

According to the method described by Phillips and Hayman, (1970).The adventitious and lateral root colonized by *G. mosseae* were collected and evaluated microscopically followed with clearing of roots in KOH 10% and staining with trypan blue 0.05% in lactophenol

Scanning electron microscope (SEM):

The study was conducted at the University Putra Malaysia /Institute of Bioscience. The root samples were cut into one mm^3 slices; each sample were covered singly with fixative solution (4% Glutaraldehyde) for 12-24 hours at 4°C. The samples were washed with 0.1 M sodium cacodylate buffer for three changes of 30 minutes for every change. Osmium tetroxide 1% was used for post fixation for two hours at 4°C. The samples were washed once more with 0.1 M sodium cacodylate buffer for three changes of 30 minutes each change. For dehydration process, samples was placed in 35% acetone for 30 minutes, followed by 50%, for 30 minutes, 75% for 30 minutes, 95% acetone for 30 minutes and finally three changes of 100% acetone at one hours interval. The samples were exposure to the critical drying point by transferring the specimens to sample basket, afterward put into a critical dryer for about 1hr. The specimens were staked onto stab using colloidal silver. The samples were coated with gold in sputter coater machine, and it was viewed using scanning electron microscope (SEM).

RESULTS

The aim of using SEM was to detect the structures of *Glomus mosseae* in chilli cell (vesicles, arbuscular, mature spore, hyphae). From our observations, we confirm that the mycorrhizal was able to colonize the cortical cell of chilli root in dual inoculation treatment and with *Glomus mosseae* alone, the *Glomus mosseae plus Actinomycetes* and mycorrhizae alone showed the best performance in all parameters. In colonized root cell, the mycorrhizae structures appear large and lignified cell wall (Figure 1). The arbuscular coil was observed in SEM Coil Cortical region colonized by Coil, and arbuscular (AR) were observed. In the current study, the structures of mycorrhizae were clearly shown using SEM. In this work, the hyphae (Hy) of mycorrhizal reach the root surface due to the signaling process, and it adhered firmly to the outer epidermal cell walls resulting in penetration of the root cells (Figure1, C).

The colonization of mycorrhizae occurs through the hyphae structure between root epidermal cells and the vast number of nuclei. The Nucleus is observed in colonized cells and to the new entry point in the cell wall. The entire arbuscular (AR) was observed surrounded by the plasmalemma of the cell host. In colonized root cell, the mycorrhizae structures appear large and lignified cell wall. The particular structures of *G. mosseae* were shown under the SEM field. In this study vesicles (V) was observed in the SEM images. The arbuscular are abundantly branched structures formed inside the cortical cell wall of the host (AR) were clearly observed using SEM. Also, we were showed in (Figure 1, B) The break roots and damage as a result of being infection by fungi. And we can also note the difference between the form root hairs (Rh)(Figure1,A), the hyphae of AMF(Figure1,C), the hyphae of the pathogen (Hp), and the hyphae of Actinomycetes(HAc)(Figure D). in Figure(F) we was showed that the colonization of mycorrhizae and Actinomycetes and did not show any symptoms on the plant by the pathogen, it same colonization in Figure (E).

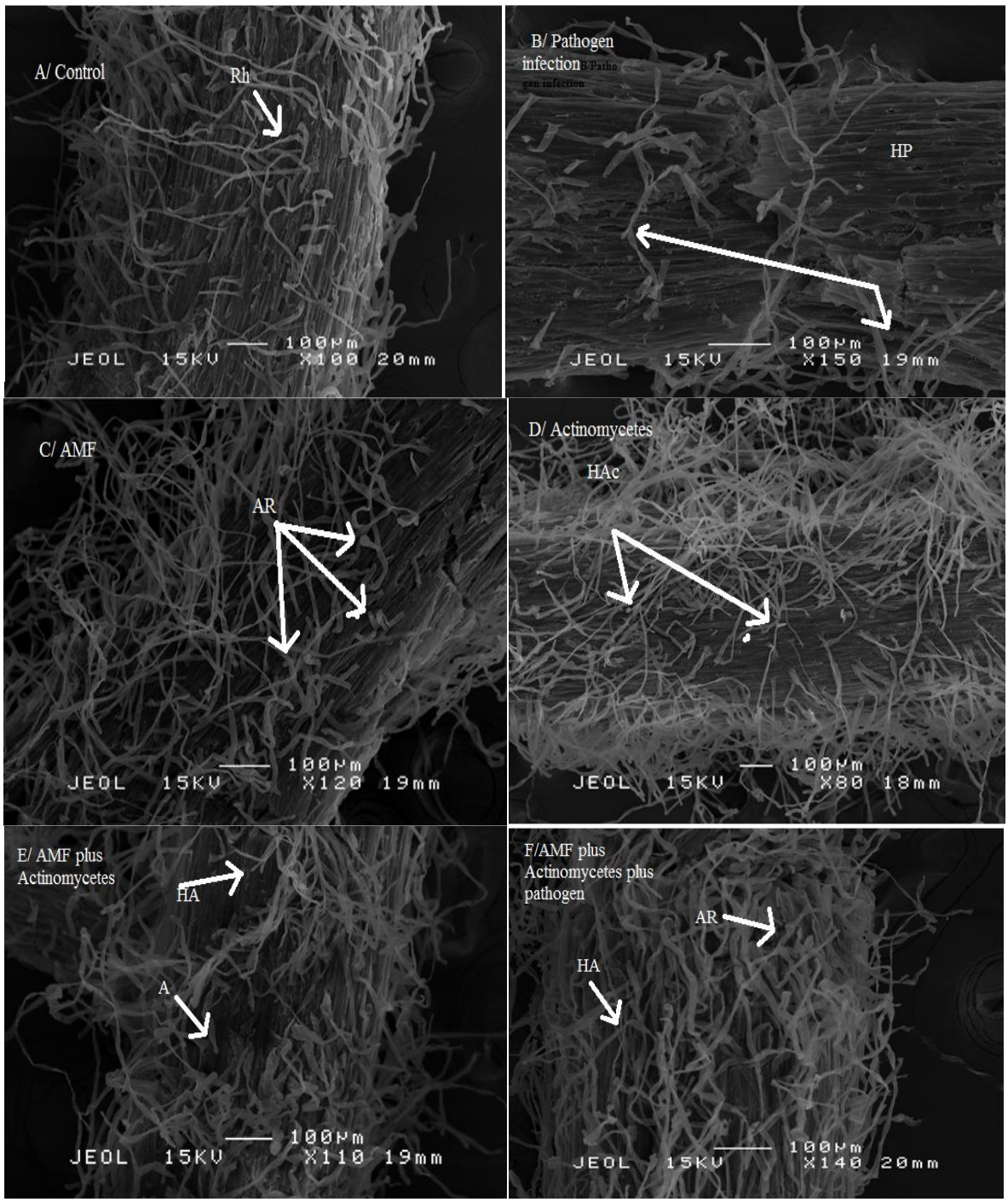

Figure 1: Ultrastructural features of *G.mosseae* in chilli root (SEM). Root hairs (Rh)(FigureA), the hyphae of AMF (AR)(FigureC), hyphae of the pathogen (Hp), and the hyphae of Actinomycetes(HAc)(Figure D).

DISCUSSION

Current studies showed that *G. mosseae* can colonize the chilli root comprehensively (Tahat *et al*., 2008; Trotta *et al*., 1996). In the current study, the structures of *Glomus mosseae* was apparently shown using Scanning electron microscope. In this study, the hyphae of *G. mosseae* reach the root surface due to the signaling process, and it adhered firmly to the outer epidermal cell walls resulting in penetration of the root cells (Figure. 1). The research done by Nicholson and Epstein (1991) was documented supporting our hypothesis; they found that the hyphae of *Fusarium oxysporum* adhered to outer epidermal cell walls during fibrillar materials and this resulted in increased the ability of the fungus to penetrate the cell causing the infection. The structures documented in this study were included, arbuscular, intra-structural coils, intercellular

hyphae. This results were agreed with the findings of Yawney and Schultz (1990) and Eduardo *et al*. (2003) they found that the general appearance of mycorrhizae structures (extraradical hyphae, intercellular hyphae & arbuscular) were detected in the root of *Anadenanthera peregrine* (L.) Spec. var. *falcate* (Benth.) and *A. peregrine* var. *facade*. The detection of the beneficial effect on chilli growth by *G. mosseae* suggest that this result is not related to enhanced plant nutrition as description by Lemanceau, (1992). Root structures modifications type, especially cell wall, cortical cell, endoplasmic reticulum, and plasmalemma suggest that the fungus can play a huge role in all root aspects. Mycorrhizae penetrate the root and grow extensively between and within living cortical cells and affects many aspects of root metabolism. The observation of root cell using SEM gave proven that the *Glomus mosseae* can invade cortical cell as a result of roots colonization. The current results were matched with that found by Balestrini *et al*. (2005) Laser microdissection observations showed that changes take place in the structure of host cells noticeably on fungal colonization, because a symbiotic interface was created by a host membrane around the fungus and the deposition of Apo-plastic layer containing molecules, which are common to the host primary wall in *Glomus mosseae* treatment where nucleus was observed round shaped and in the central position. The results present in this study are in the line with the results by Berta and Fusconi (1998); they demonstrated that in mycorrhizal *Allium porrum* cv. Early Mech, nuclei are round, in the central position, and larger compared to the control treatment. The dramatic modifications of chili cell architecture were recorded in this study (Figure1).

Many researchers documented the same finding (Genre *et al*., 2009; Bonfante & Perotto, 1995).

They reported that the morphology of the nucleus, can invade the plant plasmalemma, and increase in the number of organelles is common features in AM chili roots. In conclusion, mycorrhizal fungi were able to change and modify the colonized root cell structures. The data presented in this investigate demonstrated the hypothesis that morphological changes in plant root intercellular and intracellular lead to changes in plant physiology and morphology. More researches about the root anatomy structures, the function of mycorrhizae in root cell, and the mechanisms concerned in the root characteristics modification using SEM, and other techniques are needed for mycorrhizal fungi future trend studies.

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