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Full Length Article

Ultra-Structural Cellular Changes in Tomato Roots Induced by Mycorrhizal Fungi Colonization

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ABSTRACT

The aim of study was to detect the structural modification of *Glomus mosseae* in tomato cells using Scanning electron microscopy (SEM) and transmission electron microscopy (TEM). SEM and TEM observations showed that tomato root cell walls were able to colonize intensively by *G. mosseae*. The availability of *G. mosseae* structures in huge amount was attributed to cell wall lignification. Mature spores of *G. mosseae* in addition to net of vesicles and arbuscules were shown attached with the root cell in SEM observation. Large number of small vacuoles observed as a response to the heavy colonization by *G. mosseae.* The entire arbuscules were observed by TEM surrounded by the plasmalemma of the cell host. The colonization by *G. mosseae* occurred through the hyphae structure between root epidermal cells and the huge number of nuclei. The nuclei were observed in colonized cells and new entry point in the cell wall. Endophytic *G. mosseae* penetrates root and grow extensively between and within living cortical cells and affects many aspects of root metabolism.

Key Words: Arbuscular mycorrhizal fungi; Cell wall; Arbuscules; Scanning electron microscopy; Transmission electron microscopy

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) symbiosis is the oldest (>460 million years BP) and most widespread type of *Glomus mosseae* association (Smith & Read, 2008). AMF and plant association has been shown to enhance plant growth (Tahat *et al.,* 2008), provide greater resistance to plant diseases (Linderman, 1994; Oseni *et al*., 2010), water deficiency conditions (Ndiaye, 2001) and salt stress (Zou, & Wu, 2011).

The mycorrhizal structural characteristic has been documented extensively (Peterson & Bonfante, 1994). In the AMF symbiosis, the fungal symbiont colonizes root cortical cells, where it establishes differentiated hyphae called arbuscules (Pumplin *et al.,* 2009). During the formation of AM symbiosis the fungus penetrates the root cortical cell walls and forms haustoria- like structures (arbuscules or coils) that interface with the host cytoplasm (Barea *et al.,* 2005). The AMF structures provide an increased surface area for metabolic exchanges between the plant and the fungus (especially the highly branched arbuscules). Other AMF produce vesicles (SchiiBle, 2002), which are structures, believed to function as storage organs. It was documented that AMF strongly colonized many crops and ornamental plants such as eggplant, cucumber, pea and tomato which lead to increase plant growth and yield (Tahat *et al*., 2008; Omorusi & Ayanru, 2011). SEM and TEM are old and important tools for understanding the cellular studies of plant roots (Wills & Cole, 1978). The aim of this study was to detect the ultra-structural changes in tomato root after the colonization of *G. mosseae* using TEM and SEM techniques.

MATERIALS AND METHODS

Biological materials and growth conditions: This study was conducted at the institute of bioscience (IBS) at the University Putra Malaysia. Healthy and mature spores were isolated and collected from the pot culture. One hundred spores for 100 g dry soil were added to the pots (20 X 20 cm) and mixed well. A commercially and certified tomato cultivar was used. The seeds were surface sterilized with 90% ethyl alcohol for 10 sec, and washed with sterile distilled water. Three seeds were planted directly into the pot. Two weeks later, the seedlings were thinned to 1 seedling/pot. The pots were kept under glass house conditions (25-30C°) for 2 months to get heavily colonized roots for the study.

Colonization assessments: The adventitious and lateral root colonized by *G. mosseae* were collected and evaluated

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microscopically followed by clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol according to the method described by Phillips and Hayman (1970).

Scanning electron microscope: The study was conducted at the Institute of Bioscience/University Putra Malaysia. The root samples were cut into 1 mm^3 slices; each sample was covered separately with fixative solution (4% glutaraldehyde) for 12-24 h at 4°C. The samples were washed with 0.1 M sodium cacodylate buffer for 3 changes of 30 min for each change. 1% Osmium tetroxide was used for post fixation for 2 h at 4°C. The samples were washed again with 0.1 M sodium cacodylate buffer for 3 changes of 30 min each change. For dehydration process, samples were placed in 35% acetone for 30 min, followed by 50%, for 30 min, 75% for 30 min, 95% acetone for 30 min and finally 3 changes of 100% acetone at 1hr interval. The specimens were staked onto stab using colloidal silver. The specimens were coated by gold in sputter coater machine and it was viewed using scanning electron microscope (SEM).

Transmission electron microscope: Primary fixation washing, post fixation, washing again and dehydration series were done as in SEM specimen's preparation. The additional step inoculated infiltration of specimens was infiltrated with acetone and resin mixture, which was prepared as the following:

Acetone: Resin
(1: 1) kept for 24 h
(1: 3) and was kept for 24 h
(0: 100%) resin, kept overnight
(0: 100%) resin for 2 h.

The specimens were placed into beam capsules filled up with resin, then polymerized in oven at 60°C for 24-48 h. The final step was the preparation of thick sectioning using ultra microtome to cut 1 MM thick section. The specimens were stained and viewed using transmission TEM to observe the structure of *G. mosseae.*

RESULTS

The general structures of *G. mosseae* were shown under the SEM and TEM field. In the current study vesicles (V) was observed in the SEM images (Fig. 1-1). The arbuscules are abundantly branched structures formed inside the cortical cell wall of the host (AR) (Fig. 1-2). Mature spores of *G. mosseae* (GS), (Fig. 1-3) were clearly observed using SEM.

Nucleus (N) in colonized cells were detected in a huge number appears surrounded by the cytoplasm (Fig. 2-1). The colonization of AMF occurs through the hyphae structure between root epidermal cells and the huge number of nuclei (Fig. 2B). The Nucleus was observed in colonized cells and new entry point in the cell wall (Fig. 2-2). The entire arbuscular was observed surrounded by the plasmalemma of the cell host (Fig. 2-3). In colonized root cell the AMF structures appears large and lignifies cell wall (Fig. 2-4). Aarbuscular coil was observed in SEM Coil (AC) (Fig. 1-4). Cortical region colonized by Coil (C) and arbuscules (AR) were observed (Fig. 1-5 & 6).

DISCUSSION

Previous studies showed that *G. mosseae* can colonize the tomato root extensively (Trotta *et al*., 1996; Tahat *et al*., 2008). In the current study the structures of *G. mosseae* were clearly shown using SEM. In this work the hyphae of *G. mosseae* reach the root surface due to the signaling process and it adhered strongly to the outer epidermal cell walls resulting in penetration of the root cells (Fig. 2). The research done by Nicholson and Epstein (1991) was documented supporting our hypothesis; they found that the hyphae of *Fusrium oxysporum* adhered to outer epidermal cell walls through fibrillar materials and this resulted in enhancing the ability of the fungus to penetrate the cell causing the infection. The structures documented in this study were included, arbuscules (1-5 & 6), intra-structural coils (Fig. A. 5+6), intercellular hyphae. These results were agreed with the findings of Eduardo *et al.* (2003) and Yawney and Schultz (1990) they found that the general appearance of AMF structures (extra-radical hyphae, intercellular hyphae & arbuscules) were detected in the root of *Anadenathera peregrine* (L.) Speg. var. *falcate* (Benthe.) and *A. peregrine* var. *facata*. The detection of the beneficial effect on tomato growth by *G. mosseae* suggests that this effect is not related to improved plant nutrition as described by Lemanceau (1992). Root structures modifications type, especially cell wall, cortical cell, endoplasmic reticulum, and plasmalemma suggest that the fungus can play a great role in all root aspects.

AMF penetrates root and grow extensively between and within living cortical cells and affects many aspects of root metabolism (Fig. 2-4). The observation of root cell using SEM and TEM gave evidences that the *G. mosseae* was able to invade cortical cell as a result of roots colonization. The current results were matched with that found by Balestrini *et al*. (2005) Laser micro-dissection observations showed that changes take place in the structure of host cells dramatically upon fungal colonization, because a symbiotic interface is 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 *G. mosseae* treatment where nucleus was observed round shaped and in the central position (Fig. 2-2). The results reported 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 central position and larger compared to the control treatment. The dramatic modifications of tomato cell architecture, was recorded in this study (Figs. 2-2). Many researchers documented the same finding (Bonfante

Fig. 1: 1) Scanning electron micrograph *G. mossea* vesicles (V); 2) Scanning electron micrograph for Net of arbuscules (AR); 3) Arbuscular Coil (AC); 4) Scanning electron micrograph of mature spore of *G. mosseae* (GS); 5 & 6) Cortical region colonized by Coil (C) and arbuscules (AR)

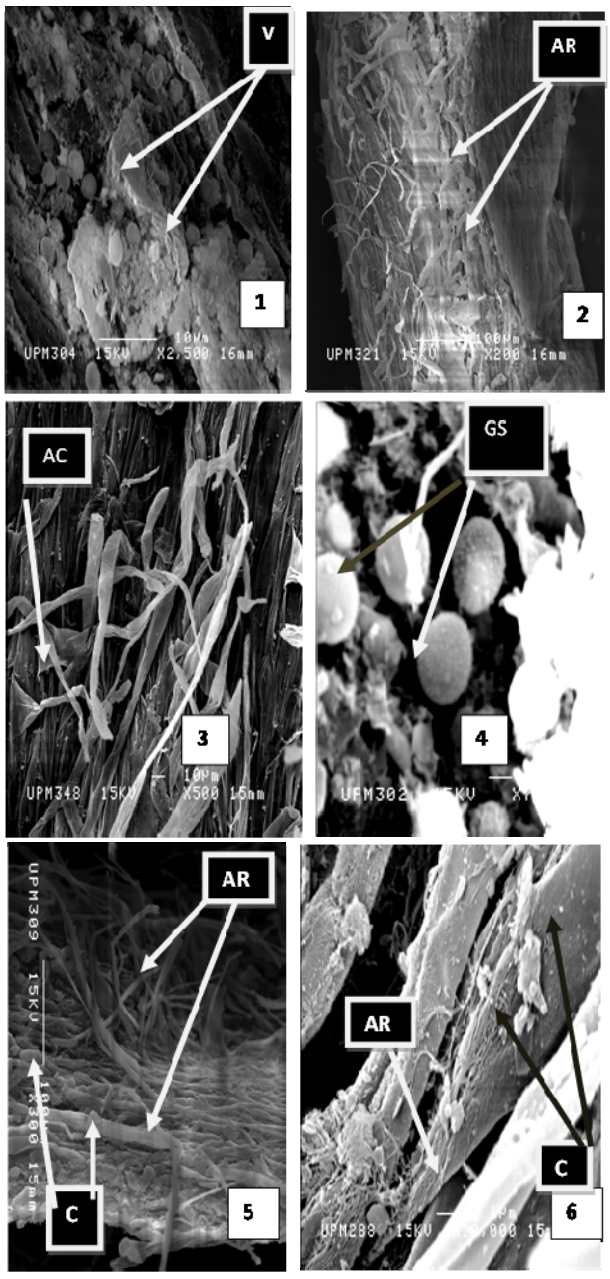
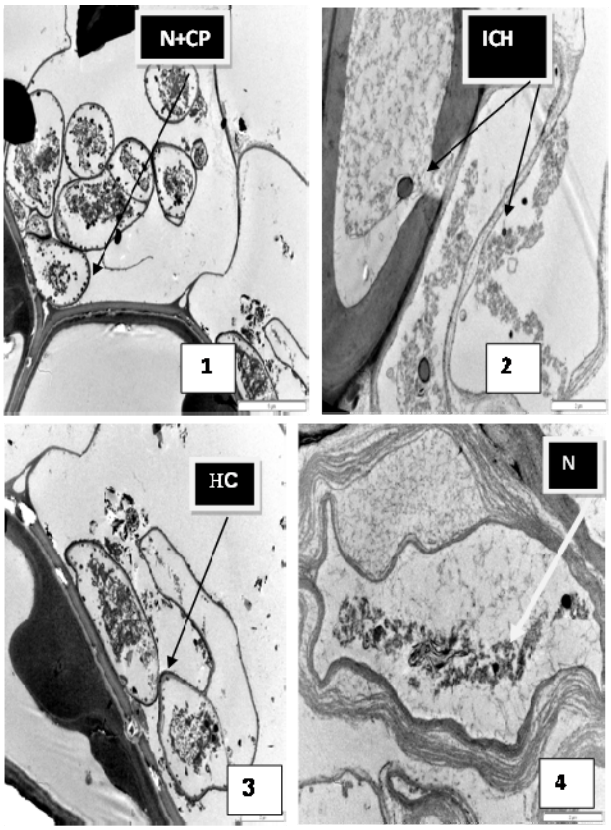


Fig. 2: Nucleus (N) in colonized cells appears surrounded by the cytoplasm (CP); (2): Intercellular hyphae penetrate into a root cell (ICH); (3): Host cytoplasm (HC) surrounding the arbuscules; (4): Nucleus (N) in colonized cells and lignified the cell wall



& Perotto, 1995; Genre *et al*., 2008). They reported that the position and morphology of nucleus, can invade the plant plasmalemma, and increase in the number of organelles are common features in AM tomato roots.

In conclusion, mycorrhizal fungi can change and modify the colonized root cell structures. The data presented in this study 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 AMF in root cell, and the mechanisms involved in the root characteristics modification using SEM, TEM and other techniques are needed for mycorrhizal fungi future trend studies.

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