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Full Length Research Paper

Ultrastructural changes of tomatoes (*Lycopersicon esculentum*) root colonized by *Glomus mosseae* and *Ralstonia solanacearum*

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The colonization of plant root cell by mycorrhizal fungi is one of the mechanisms involved for the understanding of plant bio-protection against soil-borne pathogens. The aim of current study was to investigate and describe tomato (*Lycopersicon esculentum*) root ultra-structural modifications caused by *Glomus mosseae* and the bacterial wilt *Ralstonia solanacearum*. In scanning electron microscopy (SEM) observations, the root cells presented several arbuscules and mature spores of *G. mosseae*. In transmission electron microscopy (TEM) observations, many entry points on the cell wall were detected in addition to nucleus, cell organs and many mitochondria. The results evidenced that the presence of *G. mosseae* can change the root architecture dramatically. *R. solanacearum* was inhibited by the endophytic fungi. *G. mosseae* structure can help the plant to prevent the pathogen bacterial invasion totally due to root architecture system changes.

Key words: Mycorrhizal fungi, bacterial wilt, tomato, root cell, scanning electron microscopy, transmission electron microscopy.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) can acts as a bio-protectional effect against soil-borne diseases (Harrier and Watson, 2004; Akkopru and Demir, 2005; Tahat et al., 2010) as well as being able to play a great role in nearly all physiological aspects of the host (Hayman, 1983). AMF improves plant bio-fertilizer (Tahat et al., 2008c), helps in phytoremediation (Mathur et al., 2007; Ngakou et al., 2007), plant nutrition support (Mahmood and Rizvi, 2010), and salt stress (Shokri and Maadi, 2009). Safir (1968) was the first to report the study on interaction of plant pathogenic fungi and species of AMF, followed by many reports confirming the reduction of disease severity as a result of AMF (Sharma and Johri, 2002).

Mycorrhizal fungi biodiversity is affected by the soil types (sandy, sandy loam and loam soil) and plant species (*Catharanthus roseus*, *Ocimum* species and *Asparagus racemosus*) (Gaur and Kaushik, 2011; Tahat et al., 2008a, b). The major challenges for the mycorrhizologist are the determination of fungus-signaling mechanisms and the understanding of arbuscular mycorrhizal colonization process (Gadkar et al., 2001). The nature biotrophic of fungi obligation has reflected the harmonious symbiotic relationship (Williams, 1992). *Glomus mosseae* induces systemic resistance against *Phytophthora parasitica* in tomato (*Lycopersicon esculentum*) tissue the cellular and molecular plant defense reactions are associated with this resistance as well as that of arbuscule-containing cortical cells (Cordier et al., 1998). The forming of hyphae, coils and arbuscules are the results of mycorrhizal fungi colonization for the root cortex intercellularly and intracellularly (Genre et al., 2005). The living arbuscules considered as the site of transfer between endophytic fungi and the host implies that the process is an active one (Yawney and Schultz, 1990). Arbuscules formed were branched structures inside the host root cortical cell wall, and its protoplast was branched outside (Gross et al., 2003). Host plasmalemma invigilates and proliferates around the developing fungus (Bonfante and Perotto, 1995). The

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preferential site for plant-fungal nutrient exchange depends on branched structures such as hyphae, coils and arbuscules (Smith and Smith, 1990). The aim of this study was to evaluate the anatomical changes in tomato root structure due to the colonization by *G. mosseae* and *Ralstonia solanacearum* using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

MATERIALS AND METHODS

Biological materials and growth conditions

G. mosseae spores were taken from the laboratory of Soil Microbiology, Faculty of Agriculture, Universiti Putra Malaysia. The spores were re-cultured in glasshouse for three months and stored under laboratory conditions at 15 to 20°C. Wet sieves technique was used to isolate and purify the AMF spores (Phillips and Hayman, 1970). Mature and healthy spores were isolated and collected from the pot culture. 100 spores for 100 g dry soil were added to the pots (20X20 cm) and mixed well with the soil before tomato planting.

A commercially, recommended, and certified tomato cultivar was used. The seeds were surface sterilized with 90% ethyl alcohol for 10 s, and washed with sterile distilled water. Three seeds were planted directly into the pot. Two weeks later, the seedlings were thinned to one seedling/pot. The plants were kept under glasshouse conditions at 25 to 30°C ± 2. *R. solanacearum* was re-cultured using casamino acid peptone glucose (CPG) media described by Cuppels (1978). Suspension of *R. solanacearum* was prepared at concentration of $10^7 mL^{-1}$ colony forming unit (CFU) and inoculated onto the tomato roots at 30 days after planting.

Colonization assessments

The percentage of adventitious and lateral root colonized by AMF was evaluated microscopically followed by clearing of roots in 10% potassium hydroxide (KOH) and staining with 0.05% trypan blue in lactophenol according to the method described by Phillips and Hayman (1970). The following formula was used to calculate the root colonization (Giovannetti and Mosse, 1980).

$$\text{Root colonization (\%)} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

The colonization percentage for the treatments was as follow:

1. *G. mosseae* (90%)
2. *G. mosseae* + *R. solanacearum* (70%)

Scanning electron microscope

The root samples were cut into 1 mm^3 slices; each sample was covered separately with fixative solution (4% glutaraldehyde) for 12 to 24 h at 4°C. The samples were washed with 0.1 M sodium cacodylate buffer for three 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 three 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 three changes of 100% acetone at 1 h interval (Scannerini and Bonfante, 1983). The samples were subjected to the critical drying point by transferring the specimens into specimen basket, then put into a critical dryer for about 1 h; the specimens were staked onto stab using colloidal silver. The specimens were coated by gold in sputter coater machine and it was observed using 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 different volumes of acetone and resin mixture. The first volume (1:1 volume) was kept for 24 h. The second volume was 1: 3 and was kept for 24 h; the third volume was 100% of resin, and was kept overnight, and the last volume was also 100% of resin and was kept for 2 h.

The specimens were placed into beam capsules filled up with resin, and then were polymerized in oven at 60°C for 24 to 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 TEM to observe the cell structure of tomato plant.

RESULTS

Ultra-structural results

The presence of typical hyphae and vesicles of AMF have been observed in the roots of various members of the solancae (Read and Smith, 2008). The aim of using SEM and TEM was to detect the structures of *G. mosseae* in tomato cells (vesicles, arbuscules, mature spore and hyphae). The images for *G. mosseae* show vesicles (V) attached with the arbuscules (AR) seen by SEM (Figure 1A). Small vacuoles were observed as a response of extensive colonization of tomato by *G. mosseae* (Figure 1B). Net of fungi arbuscules (AR) and different sizes of vesicles (V) were observed in the SEM images (Figure 1C); mature spore of *G. mosseae* (GS) (Figure 1D) was observed and huge number of nuclei was also observed by TEM (Figure 2A). Entry points of AMF in cell wall were detected clearly (Figure 2B) and plasmalemma of tomato cells was recognized (Figure 2C) while *G. mosseae* penetrated root and grow within and between cortical cells (Figure 2D).

DISCUSSION

G. mosseae is one of the endomycorrhizas fungi, which can enter host cortical and epidermal cells (Brundrett, 2002). This agrees with the results obtained from presented study, that the root of *G. mosseae* treatment showed the best performance in considered parameters. The current observations of SEM and TEM confirmed that the *G. mosseae* was able to colonize tomato cortical cells in the complex inoculation treatment (*G. mosseae* + *R. solanacearum*) (Figure 1A). The same observation

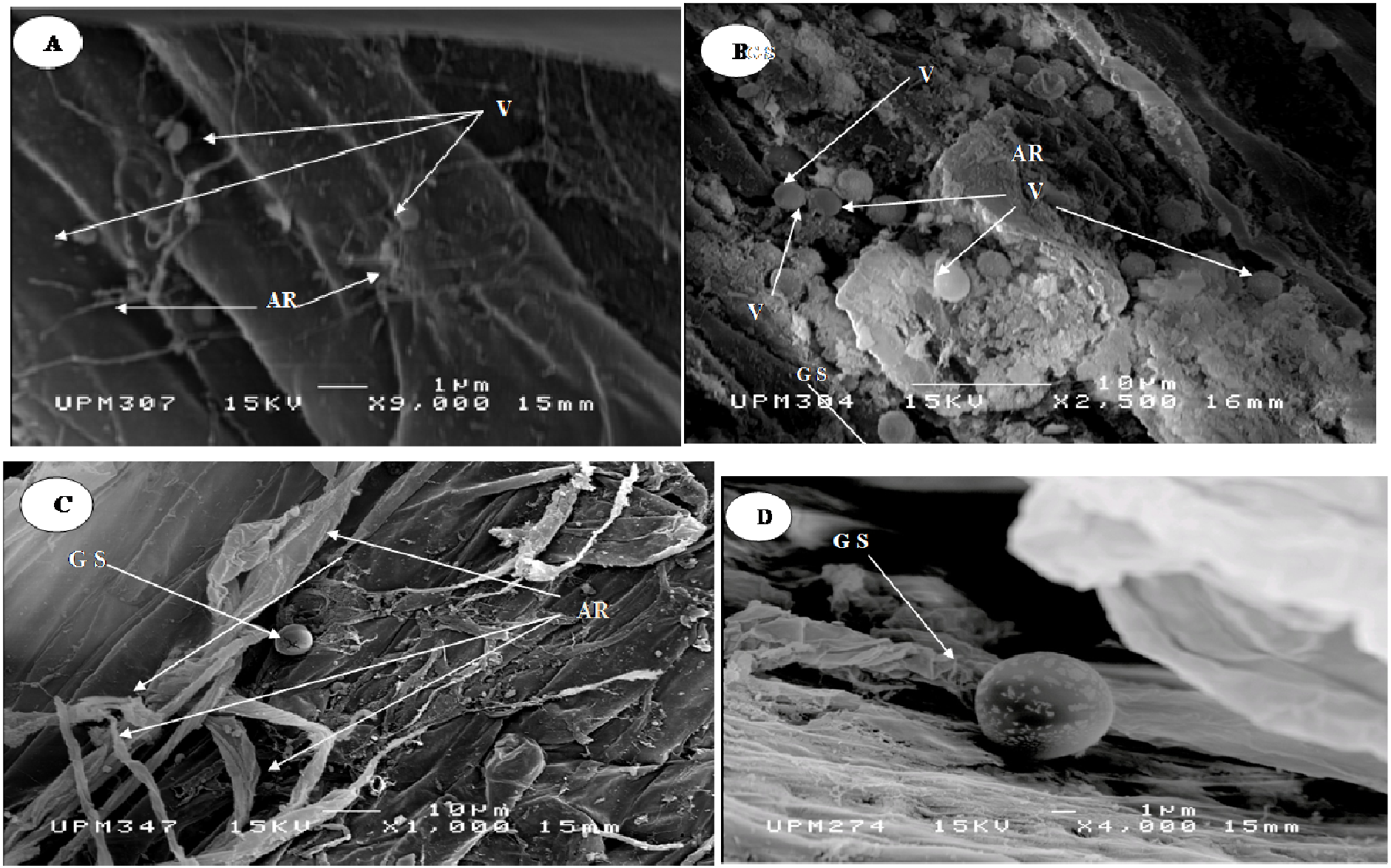

Figure 1. Ultrastrutural features of *Glomus mosseae* in tomato root (SEM). (*G. mosseae* + *R. solanacearum* treatment): A) *G. mossea* vesicles (V) attached with the arbuscules (AR) seen by SEM; B) large number of small vacuoles observed as a response to the heavy infection by *G. mosseae;* C) net of fungi arbuscules (AR) and vesicles (V); D) scanning electron micrograph (SEM) of mature spore of *G. mosseae* (GS).

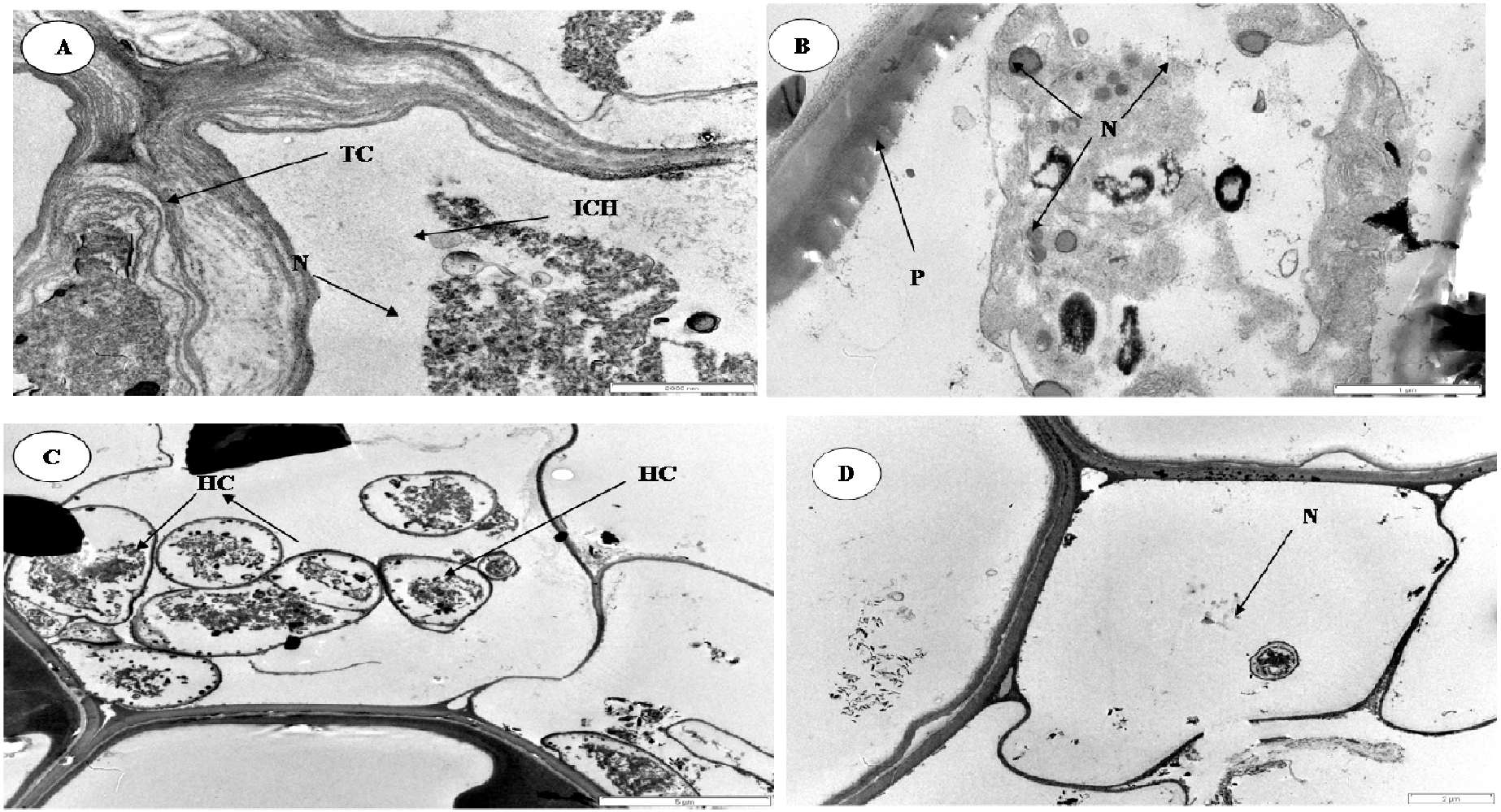

Figure 2. Ultrastrutural features of *Glomus mosseae* in tomato root (TEM). *G. mosseae* + *R. solanacearum* treatment. A) ICH, Intercellular hyphae penetrate into a root cell; TC, thicker wall induced by the penetration of AMF, huge number of nuclei (N) surrounding vesicles of *G. mosseae*; B) N, nucleus in colonized cells, new entry point in the cell wall (P); C) HC, host cytoplasm surrounding the arbuscules, it is rich in organelles, plastids, free ribosomes and rough endoplasmic reticulum. The thickness of the cytoplasmic varies from 0.50 nm upwards. D) N, nucleus in colonized cells (it appears larger than in uninfected cells). AMF, Arbuscular mycorrhizal fungi.

was obtained by Fusconi et al. (1999), who reported that *G. mosseae* vesicles were attached with the arbuscules seen by SEM. Large number of small vacuoles were observed as a response to the heavy colonization by *G. mosseae* (Figure 1B), these vacuoles act as a viable propagules

and storage structures (Plenchette and Strullu, 2003). The current results are closed to that reported by Wagg et al. (2011) who found that *Glomus intraradices* produced large numbers of vesicles in pine (*Pinus aphremphous*) forest tree.

Net of arbuscules and vesicles V was observed in the SEM images (Figure 1C). The research conducted by Mahmood et al. (2004) recorded some images closed to the images observed in this study which illustrated the arbuscules and vesicles in huge number. Many researchers observed mature spores of several mycorrhizal species using SEM, for example, Roesti et al. (2005) observed *Glomus geosporum* and *Glomus constrictum.* Blaszkowski et al. (2010) observed *Glomus africanum* and *Glomus iranicum* and Blaszkowski et al. (2006) observed *Glomus drummondii* and *Glomus walker*. In the current study, the Glomus spores was clearly observed by SEM (Figure 1D).

The anatomical and morphological changes due to the colonization of *G. mosseae* around tomato root were reported by Tahat et al. (2008c) who found that *G. mosseae* was able to increase root volume, length, size and weight. The clear effect of *Verticillium dahliae* on root cortex colonized by AMF suggest competition between AMF and the pathogen for host resources and/or space (Garmendia et al., 2005). In the current study, the inhibition of *R. solanacearum* was significantly observed, that is, no structures for the pathogen was shown in the complex treatment (*G. mosseae* + *R. solanacearum*) (Figure 1D). Mycorrhizal fungi structures were found in plenty amount which cause lignifications for the cell wall (Figure 1A and B). The infection of AMF occurs through the hyphae structure between root epidermal cells and the huge number of nuclei (Figure 2A). The nuclei were observed in colonized cells and new entry point in the cell wall (Figure 2B). The entire arbuscular was observed to be surrounded by the plasmalemma of the host cell (Figure 2C). AMF penetrates root and grow extensively between and within living cortical cells and affects many aspects of root metabolism (Figure 2C). SEM and TEM root cell observations suggested that *G. mosseae* was able to protect host cell from the invasion of *R. solanacearum* as a result of tomato roots colonization. The current results correlate with those reported by Cordier et al. (1998), who proposed that the AMF *G. mosseae* has the ability to confer bio-protection against *Phytophthora parasitica* in tomato roots. *G. mosseae* treatment nuclei where observed in round shape and in the central position (Figure 2B). The results reported in this study are in 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 host cell architecture such as position and morphology of nucleus, invagination of the plant plasmalemma, and increase in the number of organelles are common features in tomato roots arbuscular mycorrhizal (AM) symbiosis (Bonfante and Perotto, 1995; Genre et al., 2008). Finally, *G. mosseae* in symbiosis with tomato plant was able to inhibit infection by *R. solanacearum* totally hence, no structures belonging to the pathogen was observed in the complex treatment (*G. mosseae* + *R. solanacearum*). The study observations matched with those reported by Carlsen et al. (2008), who documented that *Pythium ultimum* was totally prevented due to the colonization of clover plants cv. Sonja by *G. mosseae*. Finally, the present study concluded that the presence of mycorrhizal fungi benefits tomato plant protection against *R. solanacearum* and enhancing plant growth and developments. The current report results support the hypothesis that morphological changes in host root intercellular and intracellular lead to changes in plant health and resistance against soil borne diseases. More researches about the root anatomy structures using SEM and TEM technique are required for mycorrhizal future trend studies.

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REFERENCES

Akkopru A, Demir S (2005). Biological control of fusarium wilt in tomato caused by *Fusarium oxysporum* f.sp. lycopersici by AMF Glomus intraradices and some rhizobacteria. J. Phytol. Pathol. 153: 544-550.

Berta G, Fusconi A (1998). Effects of arbuscular mycorrhizal and ericoid fungi on the structure and activity of host cell nuclei. In: plant cell proliferation and its regulation in growth and development, Bryant JA and Chiatante D (Eds.). John Wiley and Sons, Chichester, UK., pp. 135-152.

Blaszkowski J, Kovacs GM, Balazs TK, Orlowska E, Sadravi M, Wubet T, Buscot F (2010). *Glomus africanum* and *G. iranicum*, two new species of arbuscular mycorrhizal fungi (Glomeromycota). Mycologia, 102: 1450-1462. http://www.ncbi.nlm.nih.gov/pubmed/20943558?dopt=Abstract PMID:20943558 [PubMed - indexed for MEDLINE.

Błaszkowski J, Renker C, Buscot F (2006). *Glomus drummondii* and *G. walkeri,* Two new species of arbuscular mycorrhizal fungi (Glomeromycota). Mycol. Res. 110: 555-566. http://www.sciencedirect.com/science/article/pii/S0953756206001225 doi:10.1016/j.mycres.2006.02.006

Bonfante P, Perotto S (1995) Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytologist, 130: 3-21. DOI: 10.1111/j.1469-8137.1995.tb01810.x

Brundrett MC (2002) Co-evolution of roots and mycorrhizas of land plants. Tansley Rev. New Phytol.154: 275-204. http://onlinelibrary.wiley.com/doi/10.1046/j.1469-8137.2002.00397.x/abstract

Carlsen SC, Understrup A, Fomsgaard IS, Mortensen AG, Ravnskov S (2008). Flavonoids in roots of white clover: Interaction of arbuscular mycorrhizal fungi and a pathogenic fungus. Plant Soil, 302: 33-43.

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998). Cell defense responses associated with localized and systemic resistance to *Phytophthora* induced in tomato by an arbuscular mycorrhizal fungus. Mol. Plant Microbe Interact., 11: 1017-1028.

Gadkar V, David-Schwartz R, Kunik T, Kapulnik Y (2001). Arbuscular

mycorrhizal fungal colonization. Factors involved in host recognition. Plant Physiol. 127: 1493-1499. DOI: 10.1104/pp.010783. http://www.plantphysiol.org/content/127/4/1493.full.

Garmendia I, Goicoechea N, Aguirreolea J (2005). Moderate drought influences the effect of arbuscular mycorrhizal fungi as biocontrol agents against verticillium-induced wilt in pepper. Mycorrhiza, 15: 345-356.

Genre AM, Chabaud T, Timmers P, Bonfante and Barker DG (2005). Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell, 17: 3489-3499.

Genre A, Chabaud M, Faccio A, Barker DG, Bonfante P (2008). Pre-penetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. Plant Cell, 20: 1407-1420. http://www.plantcell.org/content/20/5/1407.full

Giovannetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist, 84: 489- 500.

Gross E, Cordeiro L, Caetano FH (2003). Anatomical and ultrastructural aspects of root and mycorrhizae of *Anadenanthera peregrina* (L.) Speg. var. falcate (Benth.) Altschul (Leguminosae-Mimosoideae). Rev. Brasil. Bot. 26: 515-523.

Cuppels DA, Hanson RS, Kelman A (1978). Isolation and characterization of a bacteriocin produced by *Pseudomonas solanacearum*. J. Gen. Microbiol. 109: 295-303.

Harrier LA, Watson CA (2004). The potential role of arbuscular mycorrhizal (AM) fungi in the bio-protection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Manage. Sci. 60(2): 149-157. http://www.ncbi.nlm.nih.gov/pubmed/14971681.

Hayman DS (1983). The physiology of vesicular arbuscular endomycorrhizal symbiosis. Can. Jor. Bot. 61: 944-693.

Fusconi A, Gnavi E, Trotta A, Berta G (1999). Apical meristems of tomato roots and their modifications induced by arbuscular mycorrhizal and soilborne pathogenic fungi. New Phytol. 142: 505-516.

Mathur N, Bohra JS, Quaizi A, Vyas A (2007). Arbuscular mycorrhizal fungi: A potential tool for phytoremediation. J. Plant Sci. 2: 127-140. http://ajinc.net/asci/ascidetail.php?doi=jps.2007.127.140&kw=

Mahmood I, Rizvi R (2010). Mycorrhiza and organic farming. Asian J. of Plant Sci. 9: 241-248. DOI:10.3923/ajps.2010.241.248. URL: http://scialert.net/abstract/?doi=ajps.2010.241.248

Mahmood A, Iqbal R, Qadrl R, Saeeda N (2004). Some observations on mycorrhizae of leucaena leucocephala (lam.) de wit. Pak. J. Bot. 36(3): 659-662. http://www.pakbs.org/pjbot/PDFs/36(3)/PJB36(3)659.pdf

Ngakou A, Nwaga D, Nebane CLN, Ntonifor NN, Tamo M, Parh IA (2007). Arbuscular-mycorrhizal fungi, rhizobia and Metarhizium anisopliae enhance P, N, Mg, K and Ca accumulations in fields grown cowpea. J. Plant Sci. 2: 518-529. DOI:10.3923/ jps.2007.518.529.http://scialert.net/abstract/?doi=jps.2007.518.529.

Roesti D, Ineichen K, Braissant O, Redecker D, Wiemken A, Aragno M (2005). Bacteria associated with spores of the arbuscular mycorrhizal fungi *Glomus geosporum* and *Glomus constrictum*. Appl. Environ. Microbiol. 71: 6673-6679, No. 11, Doi:10.1128/AEM.71.11.6673-6679.2005

Phillips J, Hayman DS (1970) Improved procedure for clearing roots and staining parasitic and vesicular mycorrizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55: 185-161.

Plenchette C, Strullu DG (2003). Long-term viability and infectivity of intraradical forms of *Glomus intraradices* vesicles encapsulated in alginate beads. Myc. Res. 107: 614-616. http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=153811 DOI: 10.1017/S0953756203007482.

Safir G (1968). The influence of vesicular-arbuscular mycorrhiza on the resistance of onion to Pyrenochaeta terrestris. M. Sc. Thesis, University of Iuinois, Urban.

Sharma AK, Johri BN (2002). Arbuscular Mycorrhizae. Interactions in Plants, Rhizosphere and Soils, Sharma AK, Shaxena AK and Johri BN (Eds.). Science Publishers, New Hampshire, pp. 69-96.

Shokri S, Maadi B (2009). Effects of arbuscular mycorrhizal fungus on the mineral nutrition and yield of Trifolium alexandrinum plants under salinity stress. J. Agron. 8: 79-83. DOI:10.3923/ja.2009.79.83 http://scialert.net/abstract/?doi=ja.2009.79.83

Smith SE, Smith FA (1990). Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytologist, 114: 1-38. DOI: 10.1111/j.1469-8137.1990.tb00370.x http://onlinelibrary.wiley.com/doi/10.1111/j.1469-137.1990.8tb00370.x/abstract.

Gaur S, Kaushik P (2011). Biodiversity of vesicular arbuscular mycorrhiza associated with Catharanthus roseus, Ocimum spp. and Asparagus racemosus in Uttarakhand State of Indian Central Himalaya. Int. J. Bot. 7: 31-41. http://scialert.net/fulltext/?doi=ijb.2011.31.41&org=11

Tahat MM, Radziah O, Kamaruzaman J, Kadir J, Masdek NH (2008a). Role of plant host in determining differential responses to *Ralstonia solanacearum* and *Glomus mosseae*. Plant Pathol. J. 7: 140-147. DOI:10.3923/ppj.2008.140.147 http://scialert.net/abstract/?doi=ppj.2008.140.147

Tahat MM, Kamaruzaman S, Radziah O, Kadir and Masdek HN (2008b). Plant host selectivity for multiplication of *Glomus mosseae* spore. Int. J. of Bot. 4(4): 466-470. DOI:10.3923/ijb.2008.466.470. http://scialert.net/abstract/?doi=ijb.2008.466.470

Tahat MM, Kamaruzaman S, Radziah O, Kadir J, Masdek HN (2008c). Response of (*Lycopersicum esculentum* Mill.) to Different arbuscular mycorrhizal fungi species. Asian J. of Plant Sci. 7(5):479-484. http://docsdrive.com/pdfs/ansinet/ajps/2008/479-484.pdf

Tahat MM, Kamaruzaman S, Othman R (2010). Mycorrhizal fungi as a biocontrol agent. Plant Pathol. J. 9: 198-207. DOI:10.3923/ppj.2010.198.207 http://scialert.net/abstract/?doi=ppj.2010.198.207

Wagg C, Antunes MP, Peterson RL (2011). Arbuscular mycorrhizal fungal phylogeny-related interactions with a non-host. Symbiosis, 53: 41-46. DOI 10.1007/s13199-011-0107-5 http://people.auc.ca/antunes/publications/wagg_antunes_2011.pdf

Williams PG (1992). Axenic culture of arbuscular mycorrhizal fungi. Methods Microbial. 24: 203-220. doi:10.1016/S0580-9517(08)70094-1.http://www.sciencedirect.com/science/article/pii/S0580951708700941.

Yawney BJ, Schultz RC (1990). Anatomy of a vesicular arbuscular endomycorrhizal symbiosis between sugar maple (*Acer saccharum* Marsh) and *Glomus etunicatum* Becker and Gerdemann. New Phtol. 144: 47-57.