Damage induced by root-knot nematodes and its alleviation by vesicular arbuscular mycorrhizal fungi in roots of *Luffa cylindrica*

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Abstract

The effect of vesicular arbuscular mycorrhizal (VAM) fungi inoculated with nematodes in roots of *Luffa cylindrica* (L.) Roem was studied under greenhouse conditions. Six treatments were used; viz., C = Control, T_1 = VAM only, T_2 = nematodes only, T_3 = VAM and nematodes simultaneously, T_4 = VAM fungi one week before nematodes and T_5 = VAM fungi one week after nematodes. Damage produced by nematodes was observed in T_2 and T_5, vascular tissue obliterated by root-knot nematodes. However, VAM fungi suppressed the effects of root-knot nematodes in T_4. Giant cells, egg-masses and females of nematodes were observed in T_2, T_5 and to some extent in T_3. VAM fungi spores were seen in cortical as well as in pith region of root in T_3.

Keywords: Vesicular arbuscular mycorrhiza (VAM), *Meloidogyne*, *Luffa cylindrica*, inoculum.

Cucurbits are an important part of nutritious diet worldwide and susceptible to over 200 diseases (Zitter *et al*., 1996; McGrath, 2004). Root-knot nematodes (*Meloidogyne* spp.) are one of the most economically important pests and cause an annual loss of Rs. 547.5 billions in cucurbits (Jain *et al*., 2007). *Luffa cylindrica* (L.) Roem is one of the cultivated vegetable reported as mycorrhizal (Gai *et al*., 2006). Biological control of plant diseases is one of the viable alternatives in sustainable agriculture. Beneficial soil micro-organisms such as VAM fungi have been proposed as a potential alternative to chemical control (Pozo & Azco´n-Aguilar, 2007; Shoresh *et al*., 2010).

Histopathological changes in plants due to vesicular arbuscular mycorrhizal (VAM) fungi mediated nematode suppression.VAM hyphae penetrated the epidermis and invaded the cortex which resulted in the formation of vesicles and arbuscules. The fungus *Glomus mosseae* rapidly invaded the citrus roots infested by *Tylenchulus semipenetrans* and produced vesicles as well as arbuscules before nematode invasion (O'Bannon *et al*., 1979; Sankaranarayanan & Sundarababu, 1994). In Pakistan, VAM and nematode interactions were reported by Jalaludin *et al*., (2008) and Hajra *et al*., (2009; 2013). The effect of VAM fungi inoculated with nematodes in roots of *L. cylindrica* was studied under greenhouse conditions.

Materials and Methods

Experimental treatments: The studies were designed to evaluate the efficacy and interaction of VAM fungi with root-knot nematodes in *L. cylindrica*. The root-knot nematode and VAM fungi were obtained from National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan. The experiment was laid down in randomized block design having three replications with six treatments viz., C = Control, T_1 = VAM only, T_2 = nematodes only, T_3 = VAM and nematodes simultaneously, T_4 = VAM fungi one week before nematodes and T_5 = VAM fungi one week after nematodes.

Seeds of *Luffa* were sterilized with $HgCl_2$ and then washed with distilled water. The sterilized seeds were sown into earthen pots filled with sandy-clay loam sterilized soil and irrigated after germination as per requirement of water with Hoagland solution.

VAM fungi inoculum: VAM fungal spores were raised in pots by maize as a host in green house in sterilized soil. By this method fungal spores were maintained completely free from microbial contamination. Nutrient requirements of the plant were fulfilled by Hoagland's solution, devoid of phosphorus. One month old infected host seedlings and its adhering soil containing hyphae, vesicles and chlamydospores were used as starter inoculum for experimentation.

Root-knot nematode inoculum: The isolated culture of root-knot nematode was maintained in pots by using brinjal (*Solanum melongena* L.) cv. Pusa purple as a host in sterilized soil in the green house. After 2 month of inoculation, the nematodes were extracted by harvesting brinjal roots. These harvested nematodes were used as a starter inoculum for the experiment.

Histopathological studies: Cross section slides of *L. cylindrica* were prepared from freshly harvested roots from each treatment. The sections were passed through the ascending percentage series of alcohol for dehydration of tissues using safranin, light green stains and fixed in Canada balsam.

Photographs: Photographs of sections of roots were captured using SONY 3CCD DXC-390 video camera. The camera was attached with an OLYMPUS BX 50 light microscope.

Results and Discussion

Normal anatomy of roots: Histologically the roots of *Luffa cylindrica* consists of uniseriate epidermis, with a single layer of compactly arranged thin-walled living cells. Epidermal cells produce root hairs, which are useful for absorption of water. The cortex is multi-seriate, relatively homogenous and consists of general cortex and endodermis. Endodermis is single layered, with compactly arranged barrel shaped cells. Endodermal cells are characterized by the presence of Casparian strips. Stele forms the central part of the root. The stele consists of pericycle, vascular strands and conjunctive tissue. Pericycle is the outermost part of the stele and is uniseriate and parenchymatous. Vascular strands are radial; xylem is exarch, closed type. The non-vascular tissue present between xylem and phloem strands is called conjunctive tissue.

Roots inoculated with VAM fungi only (T_1): In roots inoculated with VAM fungi only amount of vascular tissue and number of vessels increased, vessels showed formation of plates, xylem cells were conspicuous (Fig. 1 A-D).

Roots inoculated with nematodes only (T_2): Stained transverse sections of *Meloidogyne incognita*-infected roots of *L. cylindrica* plants were microscopically examined. *Luffa* roots exhibited severe infection of *M. incognita* and some alterations in the stellar and root cells were noticed when compared with that of the healthy one (Fig. 2 A-D). It was observed that when *M. incognita* invaded *L. cylindrica* roots, severe damage caused to cells (Fig. 2 A-C). Disruption of cortical cells was also observed (Fig. 2 D).

VAM + nematode (simultaneously) (T_3): In VAM + nematode (simultaneously) root sections, numerous giant cells having dense and granular cytoplasm were found in the endodermis. The giant cells were surrounded by hyper-plastic cells and they were in close vicinity to the nematode female. In some cases, multinucleated giant cells occupy a part of the stele region with displaced and deformed xylem. The nuclei were scattered or aggregated within the giant cells having boundary walls. *M. incognita* caused severe damage to roots (Fig. 2 E, F). Fungal spore formation was seen in the cortical region of root (Fig. 2 G, H). The presence of the large multinucleated giant cells in the stele with compressed and dislocated xylem and vessel elements may result in poorer sponge-gourd fruit formation and interruption of the host nutrient translocation.

Alleviation of damage of roots by VAM fungi before nematodes (T_4): Giant cells were detected in endodermis and stele regions; their size and number was smaller than in roots treated with nematodes. However, epidermis was disrupted due to nematodes. The egg-masses were detected in the cortex and endodermis (Fig. 3 B). Amount of phloem increased due to presence of VAM fungi one week before nematodes which help root to draw more nutrients and overcome the effects of nematodes (Fig. 3 A-D).

Damage of roots by nematodes before VAM fungi (T_5): Endodermis and pericycle appeared as distorted layers. The roots with early stages of infection due to nematodes one week before VAM fungi showed partial destruction of phloem; later on complete destruction was seen due to the pressure of accumulating piles of undifferentiated tissues in the infected zones. In later stages of infection, stellar part, specially phloem parenchyma, xylem parenchyma and medullary ray parenchyma was observed as the chief site of feeding with abnormal growth (Fig. 3 E-G).

Results of the present study showed reduced incidence of root-knot disease in *L. cylindrica* upon, pre-, post- and simultaneous inoculation with VAM fungi. Likewise, both lower nematode reproduction and plant growth reduction was observed upon pre, post and simultaneous inoculation of VAM fungi and the nematode *M. incognita*. Plant growth reduction caused by individual infection with the nematodes as well as nematode reproduction was always significantly lower in the presence of VAM fungi.

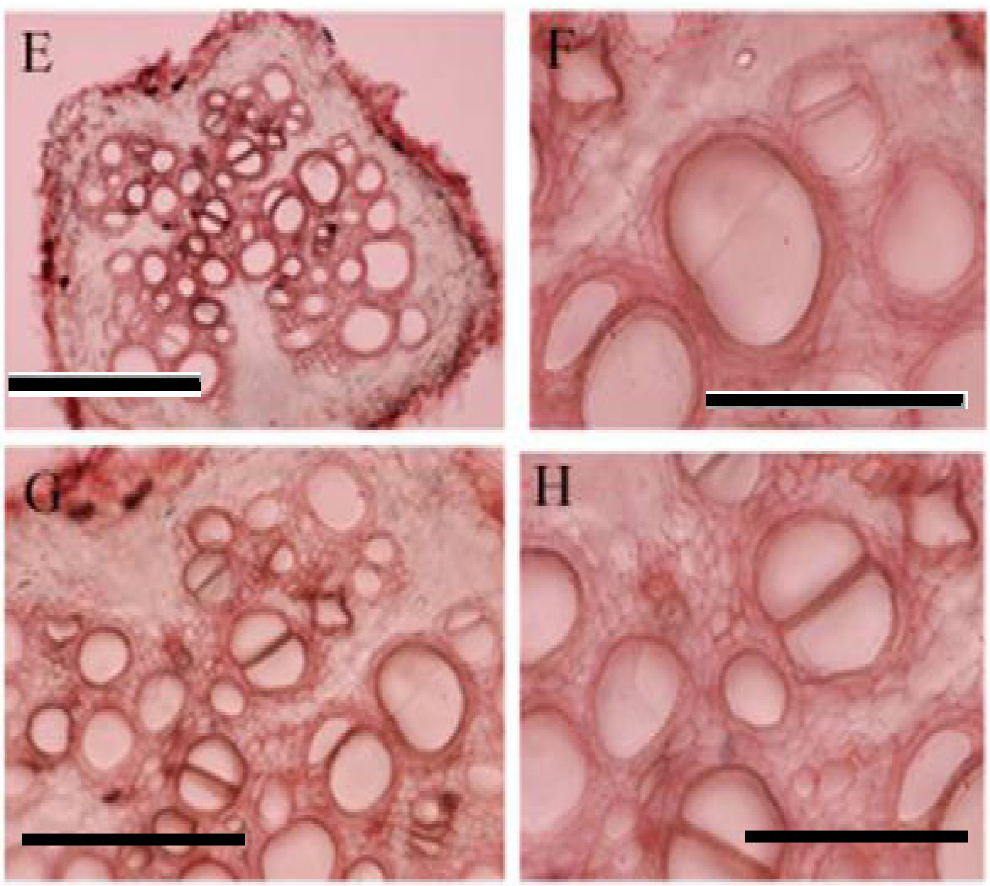

Fig. 1 (A-D). Effect of VAM on anatomy of root; A. 10x showing increased number of vascular bundles and stellar cell structure; B. 40x showing xylem cells; C-D. 20x showing large vessels.

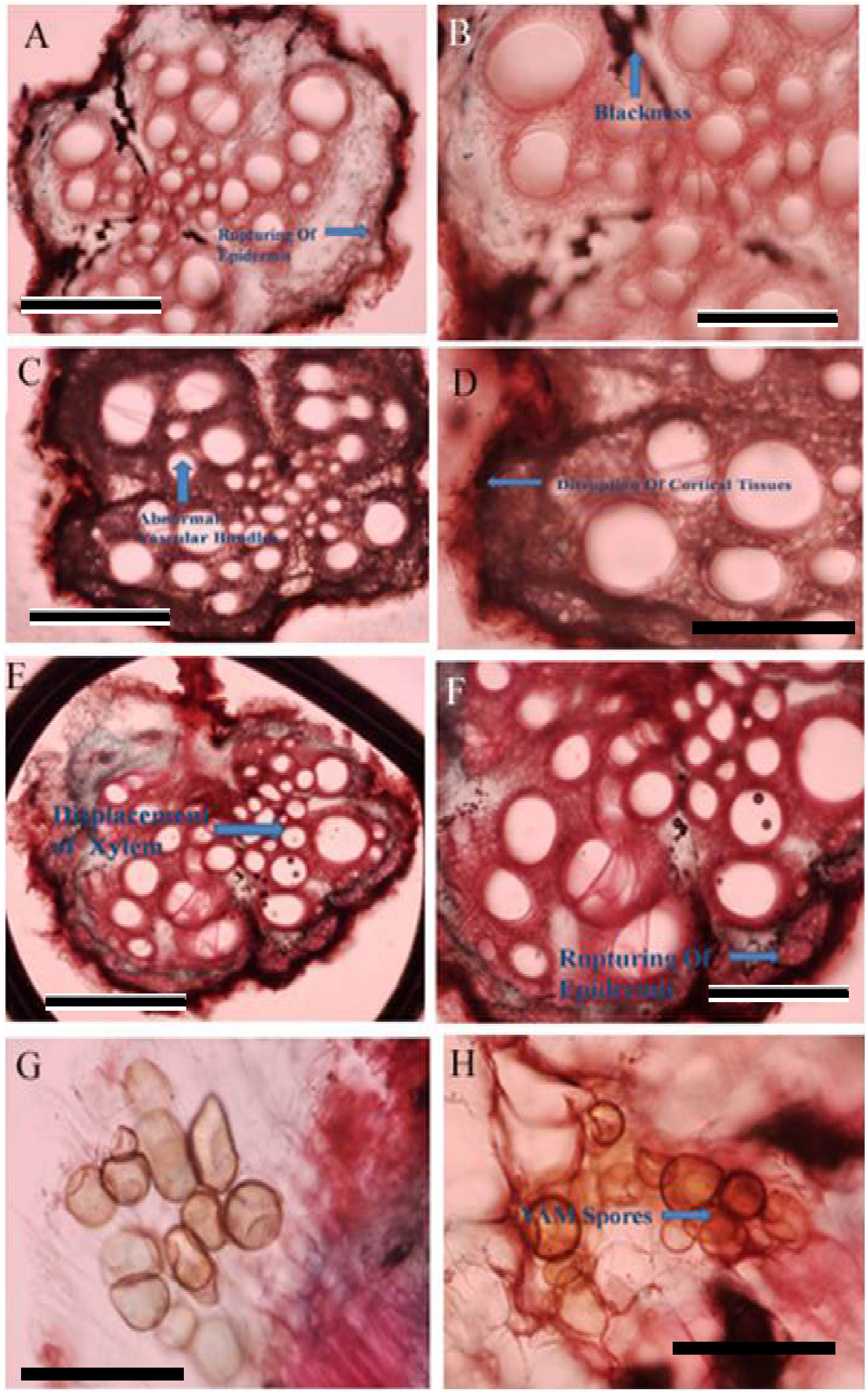

Fig. 2 (A-H). A-D: Effect of nematode on anatomy of root of *Luffa cylindrica*: A. 10x showing disruption of epidermal cells; B. 10x showing blackness in stellar region; C. 10x showing disruption of stellar cells; D. 20x showing changes in cortical cells; E-H: Effect of VAM + nematode (simultaneously); E. 10x showing displaced xylem; F. 10x showing changes in epidermal cells; G-H. 20x showing VAM spores.

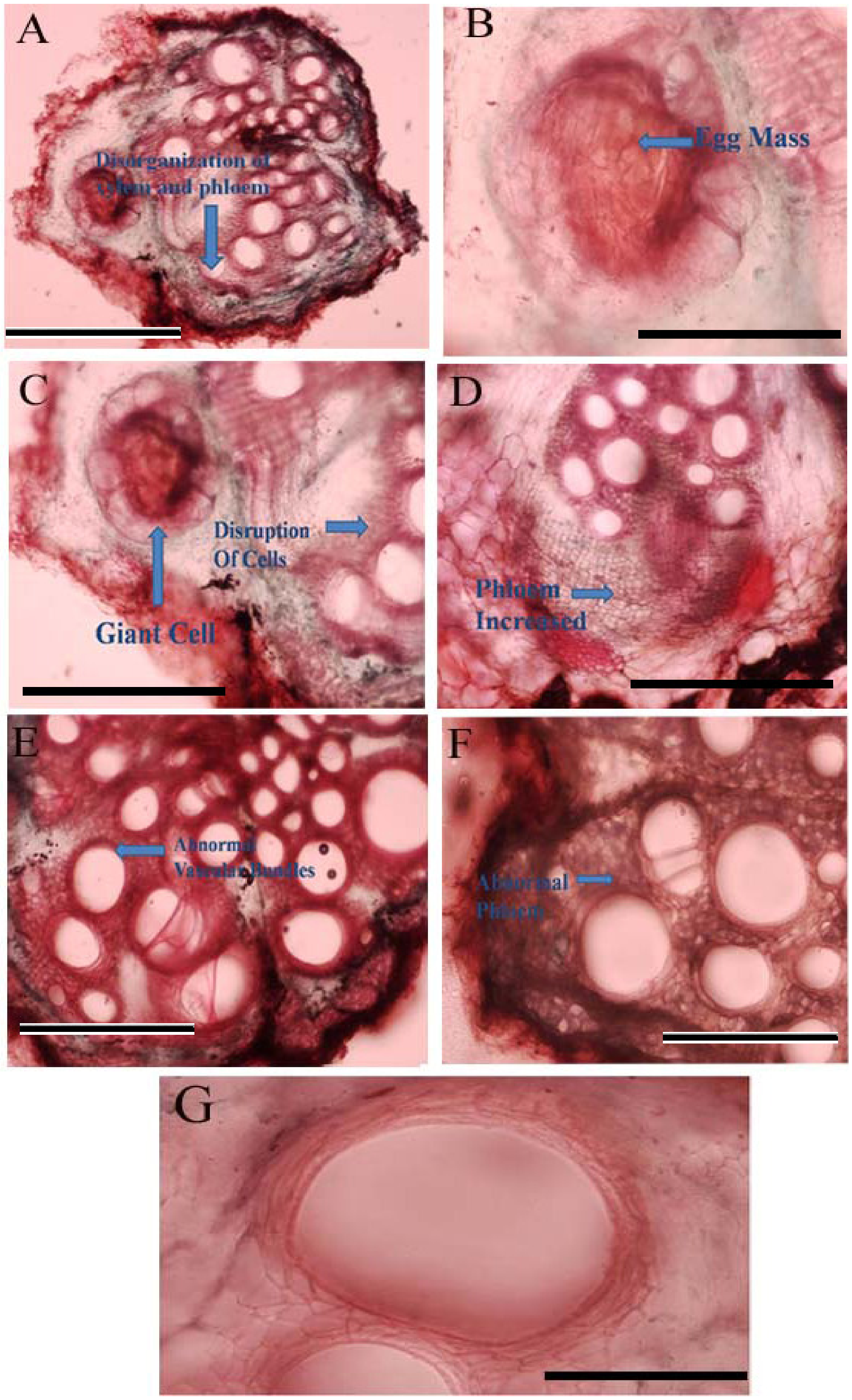

Fig. 3 (A-G). A-D: Effect of VAM + nematode (VAM one week before nematodes) on anatomy of root of *Luffa cylindrica*: A. 10x showing changes in vascular bundles; B. 20x showing egg-mass in giant cell; C. 20x showing disruption of stellar cells and presence of giant cell; D. 20x showing increased phloem; E-G. Effect of nematodes + VAM (VAM one week after nematodes); E, F. 10x showing abnormal xylem and phloem cells; G. 20x showing large vessel.

VAM fungi reduced the harmful effect of root infection by many parasitic nematodes in *Luffa* and results agreed with Hussey & Roncadori (1982), Sharma *et al*., (1994), Jothi & Sundarababu (2002) and Shreenivasa *et al*., (2007). Data obtained regarding the VAM and nematodes was also in accordance with Suresh (1980), Sikora (1981), Duchesne (1994) and Linderman (1994). Mycorrhizal plants showed less numbers and small sized giant cells than in non-mycorrhizal plants (Trudgill & Parrott, 1969; Fassuliotis, 1970). Histopathological changes observed in luffa roots were also reported in many host plants (Mahapatra & Swain, 2006; Senthilkumar *et al*., 2007).

Conclusion

It is concluded that VAM fungi caused an increase in plant growth by developing resistance to nematode infection and development, served as an effective and alternative biocontrol agent against nematode pests.

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