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RESEARCH PAPER

Histopathological Studies of Mungbean Plant Roots Inoculated with Mycorrhiza and *Macrophomina phaseolina* (Tassi.) Goid

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ABSTRACT

Mungbean dry root rot incited by *Macrophomina phaseolina* (Tassi.) Goid is the most destructive seed and soil borne disease which appears every year and causes heavy losses in yield. Present investigation was carried out in screenhouse pot condition for histopathology of mungbean plant roots (variety MH 318) inoculated with *Glomus hoi* (mycorrhiza) and *M. phaseolina* using microscopy. In the present study, three treatments inoculated with mycorrhiza, *M. phaseolina* and control plant roots were observed. Among them mycorrhizal-inoculated roots, interwoven fungal mycelium, arbuscules and vesicles were observed within cortical cells, resulting in a compacted cortex, while these structures were absent in control roots. The pericycle, located beneath the endodermis and consisting of one to two cell layers, remained unaffected by mycorrhizal colonization. The vascular bundles, containing xylem, phloem and occasional parenchymal cells, were structurally consistent in both mycorrhizal and non-mycorrhizal roots, indicating that mycorrhizal inoculation did not impact the plant's vascular transport system. In contrast, pathogen-infected roots exhibited disrupted epidermal and cortical cells. These findings contribute to understanding the structural dynamics of mycorrhizal and pathogenic interactions in mungbean roots.

HIGHLIGHTS

- Histopathological studies were understanding the structural dynamics of mycorrhizal and pathogenic interactions in mungbean roots.

Keywords: *Glomus hoi*, *Macrophomina phaseolina*, histopathology, vascular transport

M. phaseolina (Tassi) Goid. is a soil and seed-borne polyphagous pathogen that causes root rot and other rots and blights in more than 500 crop species. Dry root rot (DRR), also known as charcoal rot, can cause yield losses of 25 to 48% (Iqbal and Mukhtar, 2014). *M. phaseolina*, a necrotrophic pathogen, infects a wide range of crops, causing root deterioration, wilting and plant death in advanced disease stages (Khan *et al*. 2017). *Macrophomina* species are responsible for diseases across diverse field crops in South and Southeast Asia, including common bean (*Phaseolus vulgaris* L.), cowpea [*Vigna unguiculata* (L.) Walp], urdbean [*V. mungo* (L.) Hepper], soybean (*Glycine max* (L.) Merr.), potato (*Solanum tuberosum* L.) and cotton (*Gossypium hirsutum* L.) (Suriachandraselvan *et al*. 2005). Given the importance of these crops and their

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vulnerability to *M. phaseolina*, this study sought to conduct a histological examination. Mungbean variety roots were investigated and compared at the cellular level.

MATERIALS AND METHODS

Mass multiplication of pathogen inoculum, soil preparation and inoculation:

M. phaseolina was mass multiplied using a slightly modified method described by Kaur *et al*. (2013). In brief, 500 mL glass bottles were filled with maize grains and sand (3:1). The media was sterilized by autoclaving (15 psi, 121±1°) the bottles for 30 min, chilled and injected with mycelium agar disc (5 mm diameter) cut from the margin of an actively growing 7-day old culture of *M. phaseolina*. It was then cultured for 25 days at 28±1°. The incubated bottles were thoroughly shaken every day to ensure that all seeds were colonized uniformly and properly. Infested maize seeds were finely crushed in a grinder to act as the pathogen inoculation. Sand, soil and farmyard manure were mixed in a 4:4:1 ratio and placed in polythene bags (30 × 20 cm) tied with a rubber band. The soil bags were autoclaved for 30 min at 121° and 15 psi. Earthen pots (15 cm diameter) were chosen and disinfected by dipping in formaldehyde (4% w/v). Pots were filled with sterilized soil (1 kilogram each) and stored in the glass house. The inoculum (100 g/pot) was well mixed in the topmost 5 cm top soil of each pot 5 days before sowing mungbean seed. In all, ten surface sterilized (0.1% w/v sodium hypochlorite) mungbean seeds (variety MH 318) were sowed in each pot that had already been inoculated with the inoculum in three replications.

Maintenance of mycorrhizal fungi

Mycorrhizal cultures (*Glomus hoi*) were procured from the Department of Plant Pathology, CCS HAU, Hisar and grown on wheat and pearl millet sown in earthen pots (20 cm diameter). The pots were filled with 5 kg of sterilized river sand. In the soil's upper layer (5 cm) mix mycorrhizal suspension which contained about 450-500 spores per kg. soil. The seeds of wheat and pearl millet were sown and pots were irrigated regularly. The shoot portion of plants was cut after 90 days at soil level and left the soil in pots to air dry. The soil was crumbled and cut the rootlets into one cm segments. This soil was used as a mycorrhizal inoculum in mungbean sowing pots.

Histopathological analysis

Histopathological studies of healthy and infected roots with of mungbean were carried out by selecting plants from pots maintained under screenhouse benches. Roots were carefully removed from the pots, washed free of soil and cut into small pieces of 0.5 to 1cm length, fixed in F.A.A for 48 hrs. Fixed roots were thoroughly washed in 50% ethyl alcohol three times at an interval of 2 to 3 hrs. Then, these roots were dehydrated through graded series of tertiary butyl alcohol 50, 70, 85, 95 and 100% for 2 hrs, overnight, 1 hr, 1 hr and 1 hr, respectively. After 100% tertiary butyl alcohol again, there were two changes of pure tertiary butyl alcohol of 1 hr duration each. In the last solution enough, safranin was added to give a red tinge in order to stain the material for easy orientation during embedding and section cutting. The dehydrated specimens were infiltrated in liquid paraffin oil and embedded in paraffin wax. Paraffin blocks containing specimens were cut and trimmed to appropriate size. They were fixed to the microtome; transverse sections of both healthy and infected roots were cut in a semi-automatic Spencer rotary microtome at 12µ thickness. Ribbons cut from the blocks floated on a hot water bath. These were collected on clean glass slides smeared with Mayers albumin, warmed over the flame of a spirit lamp to stretch and fix the ribbons on slides. Serial sections on the slides were processed through xylene and alcohol. The slides were cleaned in distilled water and stained with 1% safranin for three hours. These were washed again with running tap water, then in distilled water. The slides were then treated in a series of ethyl alcohols (30%, 50%, 70% and 95%) for 5 minutes each. Serial section slides were counterstained with 0.5% malachite green for 10 minutes. They were processed through absolute alcohol (1 min) mixture containing equal parts of absolute alcohol and xylene and finally xylene 100% keeping for 5 min. in each solution. The sections were ultimately mounted on glass slides in DPX mountant covered with rectangular cover slip and dried in the oven at 45° for overnight. The slides were examined under research microscope for histopathological observation and photographs were taken.

RESULTS AND DISCUSSION

This study focused on analyzing serial transverse microtome sections of healthy, mycorrhizal-inoculated and *M. phaseolina*-infected mungbean roots cultivated under screenhouse conditions. In healthy root sections (Fig. 1), a distinct single-layered, barrel-shaped epidermis was observed, along with a multilayered hypodermis or cortex, a single-layered endodermis and pericycle. Conjunctive tissues comprised radially arranged parenchymatous cells located between the vascular bundles. The vascular bundles exhibited a radial and tetrarch arrangement, with alternating xylem and phloem. The xylem was exarch, with polygonal metaxylem vessels and a diminished pith.

In mycorrhizal-inoculated root sections, interwoven fungal mycelium was present. Arbuscules were noted within the cortical region and vesicles appeared within the cortical cells of mycorrhizal roots. The cortex in these roots displayed greater compactness compared to the control roots, which lacked arbuscules and vesicles. The innermost endodermal layer remained intact, with an unaffected pericycle layer, one to two cells thick, directly beneath. Centrally, a compact vascular bundle was noted, composed of xylem and phloem with a few parenchymal cells. These vascular structures were consistent between mycorrhizal and non-mycorrhizal roots of mungbean plants, suggesting that mycorrhizal inoculation did not disrupt the plant's vascular transport system (Fig. 2).

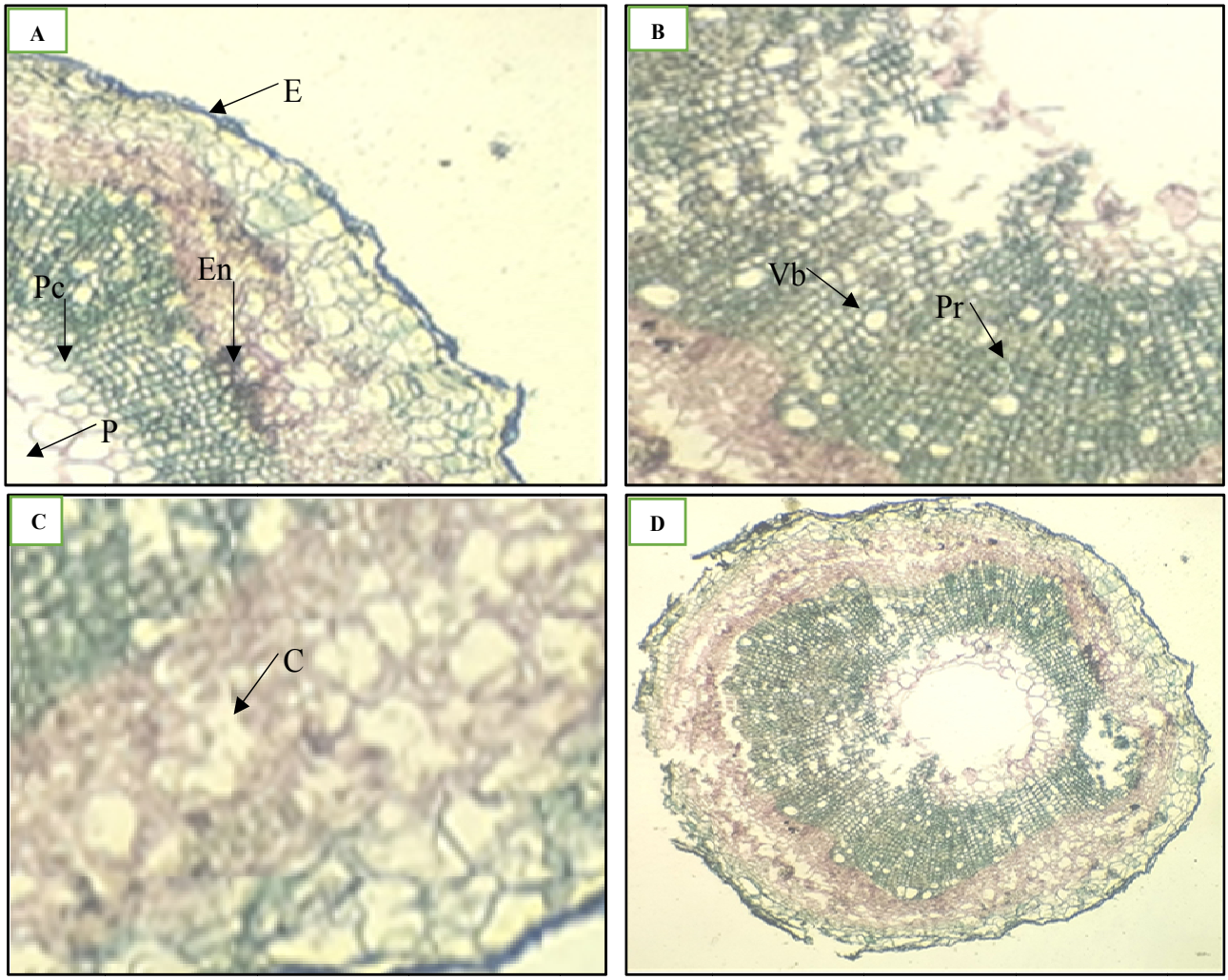

Fig. 1: Histopathology of uninoculated mungbean root parts (4x). **(A)** Epidermal region of root. **(B)** Radial arrangement of parenchymatous cells between the vascular bundles of roots. **(C)** cortex region of roots. **(D)** Transverse section of root. *E* = Epidermis, *En* = Endodermis, *Pc* = Pericycle, *P* = Pith, *Vb* = Vascular bundle, *Pr* = Parenchyma and *C* = Cortex

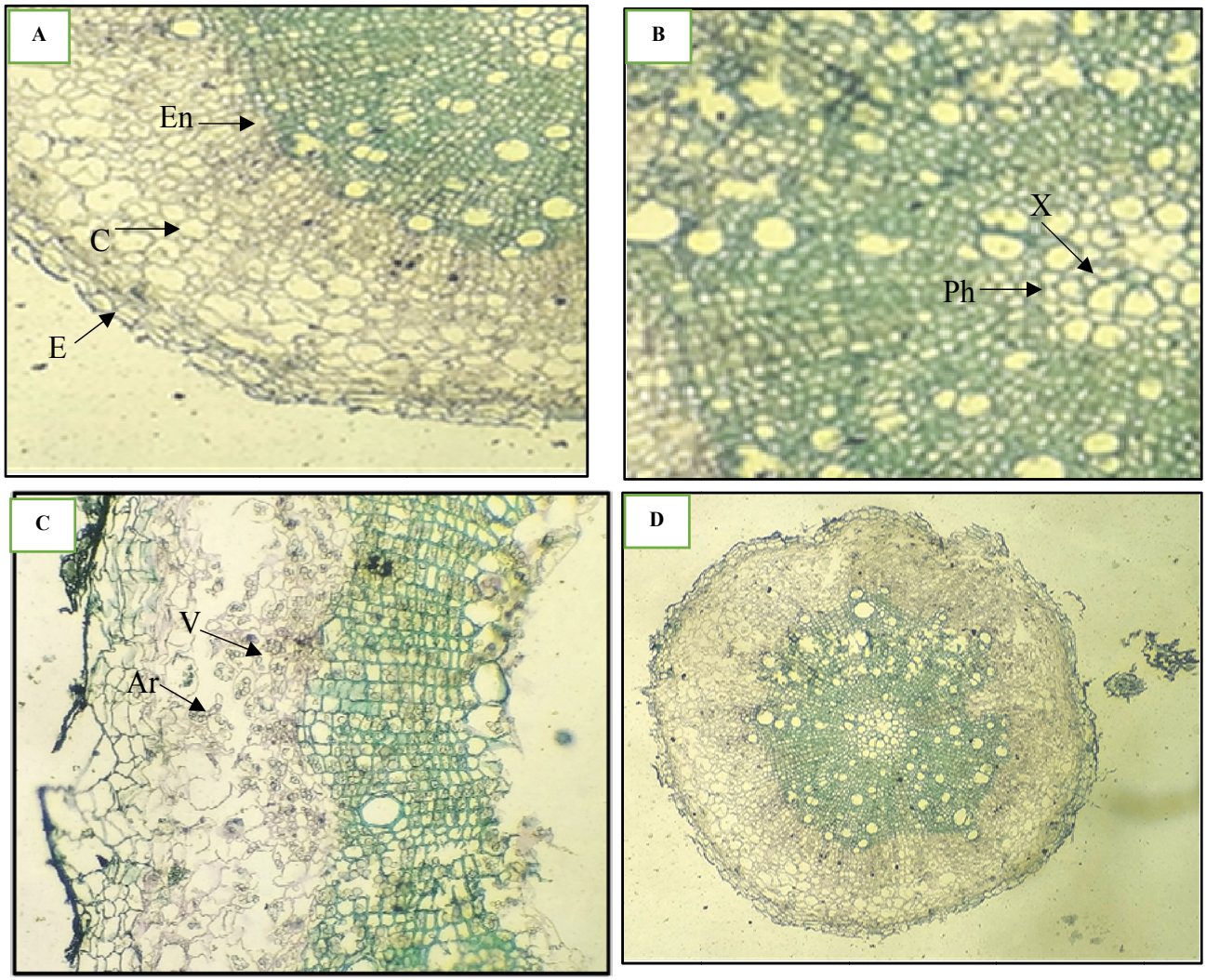

Fig. 2: Histopathology of mycorrhiza inoculated mungbean root parts (4x). **(A)** Epidermal region of root. **(B)** Radial arrangement of parenchymatous cells between the vascular bundles of roots. **(C)** cortex region of roots. **(D)** Transverse section of root. *E* = Epidermis, *En* = Endodermis, *C* = Cortex, *Ph* = Phloem, Xylem, *Ar* = Arbuscular and *V* = Vesicle

In contrast, pathogen-infected root sections showed disruptions in both epidermal and cortical cells (Fig. 3). These observations align with prior studies on the histopathology of sorghum seedling roots infected by *M. phaseolina*, which described intercellular hyphal colonization and the formation of microsclerotial bodies within cortical, xylem and phloem parenchyma tissues (Karunakar *et al*. 1992). Histopathological studies have shown direct hyphal penetration into epidermal cells, followed by intercellular migration to mesophyll tissues. Similar direct penetration has been documented in *Phoma medicaginis* on alfalfa leaves (Castell Miller *et al*. 2007), *P. clematidina* on clematis (*Clematis* spp.) leaf surfaces (Van de Graaf *et al*. 2002) and *Stagonospora nodorum* on wheat leaves (Solomon *et al*. 2006). Microsclerotia often form appressoria on host epidermal cells, from which hyphae develop and spread between epidermal cells, causing mechanical or enzymatic damage. The lamella and cell wall disintegrate, resulting in intracellular colonization (Ammon *et al*. 1974). *M. phaseolina* enters the vascular system after breaching the epidermis and cortex, generating microsclerotia on xylem vessels that can produce obstruction and wilt symptoms (Ilyas and Sinclair 1974). *M. phaseolina* is classified as a necrotrophic pathogen because of its necrotic symptoms and devastating effects on host plants (Bellaloui *et al*. 2012; Acharya *et al*. 2013). Mechanical stress, enzymatic digestion, inherent holes and ulcers all help pathogens penetrate into plant cells (Horbach 2011).

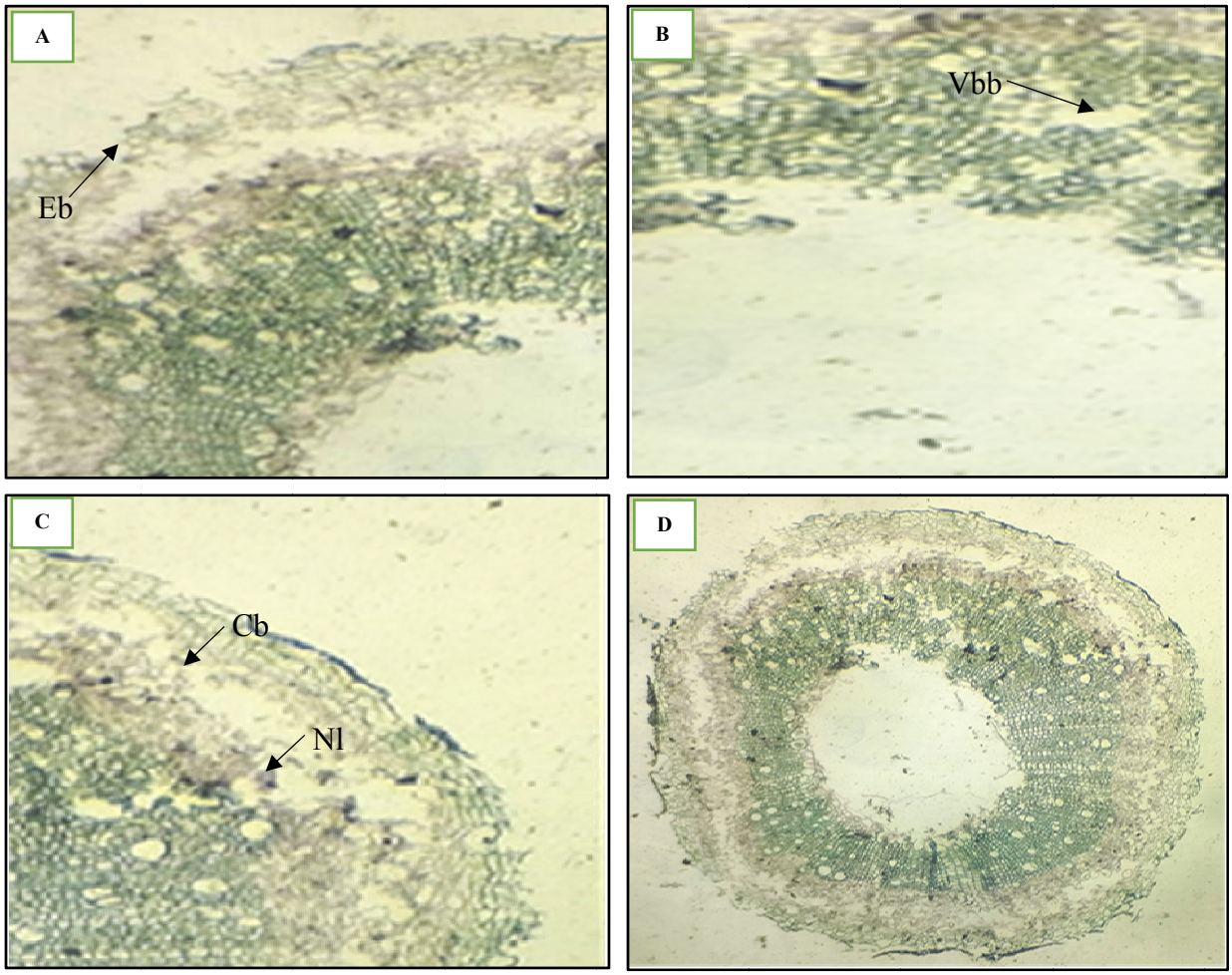

Fig. 3: Histopathology of pathogen inoculated mungbean root parts (4x). **(A)** Epidermal region of root. **(B)** Breakdown of parenchymatous cells and vascular bundles of roots. **(C)** cortex region of roots. **(D)** Transverse section of root. *Eb* = Breakdown of Epidermis, *Vbb* = Breakdown of Vascular bundle, *Cb* = Breakdown of Cortex and *Nl* = Necrotic lesion

SUMMARY AND CONCLUSION

The study investigated the histopathological differences in mungbean roots that were healthy, mycorrhizal-inoculated and infected by *M. phaseolina*. Healthy roots displayed well-organized layers with a clear vascular arrangement. Mycorrhizal-inoculated roots exhibited compact cortical regions with the presence of fungal structures like arbuscules and vesicles, but the vascular system remained unaffected, indicating that mycorrhizal colonization did not interfere with root transport functions. Conversely, *M. phaseolina*-infected roots showed severe cellular disruptions, particularly in epidermal and cortical tissues, with pathogen spread into the vascular system, leading to potential blockages and plant wilting. This pathogenic invasion process, characteristic of necrotrophic behaviour, highlights the destructive impact of *M. phaseolina*, emphasizing the need for control measures in susceptible crops.

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