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Histopathological Profiling of Banana Varieties Infected with *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4

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ABSTRACT

Fusarium wilt disease has been a major threat to the banana industry in the Philippines and efforts are of utmost importance to combat it. The present study evaluated the histopathological characterization of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 infecting two susceptible varieties, the Lakatan and Cavendish cv. Gran Naine banana seedlings. This is to understand the pathogen's aggressiveness and the host plant response mechanism. The study was conducted using histopathological profiling techniques, including pathogen isolation, pathogenicity testing, and tissue analysis. The pathogenicity test for the *Foc*TR4 isolates has demonstrated an external symptom of yellowing on the older leaves and pseudostem splitting, with an internal manifestation of corm and vascular discoloration. Histological examination of the *Foc*TR4-infected tissue exhibited massive colonization of fungal mycelia to the xylem vessels and was able to produce a chlamydospores and microconidia structure. Moreover, both varieties exhibited a defense response to the pathogen attack by the production of its phenolic compounds which is more evident in the Lakatan variety. These findings emphasize the virulence nature of the *Foc*TR4 and highlight the importance of developing resistant cultivars and integrated management strategies. Further research should focus on the molecular level in understanding the histopathological

characterization of *Foc*TR4-infected banana varieties at the nursery and field conditions.

Keywords: *histopathology, pathogenicity, virulence, FocTR4, banana varieties*

Introduction

The banana (*Musa* spp.) cultivation is an important part of global agriculture, serving as a significant source of nutrition and economic livelihood for millions of people worldwide (Bakry et al., 2009). This has driven the Philippines to become the second largest exporter of bananas globally, in terms of volume in 2022 (Rivera, 2023). The sustainability of banana production, however, is vulnerable to pests and diseases that can damage its growth and yield. Approximately 10% of the land designated for export production in Mindanao is affected by Fusarium wilt, and the disease continues to spread with varying levels of severity (ACIAR, n.d.).

The Fusarium wilt disease caused by the fungus, *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 had been discovered in the bananas of Davao region's growing area, which caused significant devastation to the Cavendish and Lakatan variety (Solpot et al., 2016). This fungal pathogen has been designated a separate species called the *Fusarium odoratissimum* and it has spread to various regions (EPPO, 2023) which produces a severe threat to banana cultivation worldwide, affecting the different cultivars (Garcia-Bastidas, 2022). According to Lumawag (2019), this problem has been the bane of the banana growers and is nowhere near to be solved for nearly two decades.

Despite research efforts, the histological manifestations of Fusarium wilt symptoms in banana varieties infected with *Foc*TR4 remain inadequately understood (Rocha et al., 2022). A histological investigation is fundamental to understanding the cellular and tissue-level alterations induced by *Foc*TR4 infection in banana plants (Garcia-Bastidas et al., 2019).

This research addresses the study of Rocha et al. (2022), which lacks information on how *Foc*TR4 affects Cavendish and Lakatan bananas at the microscopic level. By filling this gap in knowledge, the study improves the overall understanding of how plants and pathogens interact. This knowledge can then be used to develop more sustainable management strategies to mitigate the impact of Fusarium wilt in banana production. The study was conducted to evaluate the histopathological characteristics of *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 infecting Cavendish cv. Gran Naine and Lakatan banana.

Methods

The study was conducted at the House of Musa Nursery and PCR Laboratory of the University of Southeastern Philippines, Tagum-Mabini Campus, Tagum Unit, from March to May 2024. Lakatan and Cavendish cv. Gran Naine banana seedlings were used in the study. The seedlings were obtained from the House of Musa Nursery, located at Coop C, Timog, Tagum City.

Preparation of Culture Medium

The commercial Potato Dextrose Agar (PDA) medium was prepared by dissolving 39g of PDA powder per liter of distilled water. The powder was mixed with distilled water in a beaker and heated in a casserole until completely dissolved. The heated PDA was

then poured into an Erlenmeyer flask and sterilized at 15 psi for 15 minutes. After sterilization, the medium was allowed to cool and poured aseptically into sterile petri plates. The researchers then allowed it to solidify for approximately 20 minutes before being used for pathogen isolation.

Isolation and Characterization of *Foc*TR4

The *Foc*TR4 was isolated from the infected banana showing typical symptoms on the field at New Corella, Davao del Norte (Figure 1. A-C). Strands of infected pseudostem with discoloration (Figure 1. D) were cut into small sections (3-6mm long) and surface-sterilized using a 10% sodium hypochlorite (NaOCl) for one minute, then rinsed thrice with sterile distilled water (SDW). The disinfected tissue was blot-dried using sterilized tissue paper and isolated on the PDA medium. The plates were incubated and observed for mycelial growth (Figure 1. E). The cultural colony of the fungus showed a slightly raised elevation with a hairy to cottony and the color exhibited from whitish to yellow to pink shades. Mycelial growth is slightly abundant, moderately rapid growing, and has aerial growth habit (Figure 2. A-B). A microscopic examination was done to confirm the identity of the fungus (Figure 2. C-F).

Figure 1. *(A) Banana Plants Infected with Fusarium Wilt; (B) Pseudostem Splitting; (C) Vascular Discoloration of the Tissue; (D) Strand of Infected Pseudostem for Isolation; and (E) Observed Mycelial Growth of Isolated Tissue on the PDA Medium at 3 Days after Incubation.*

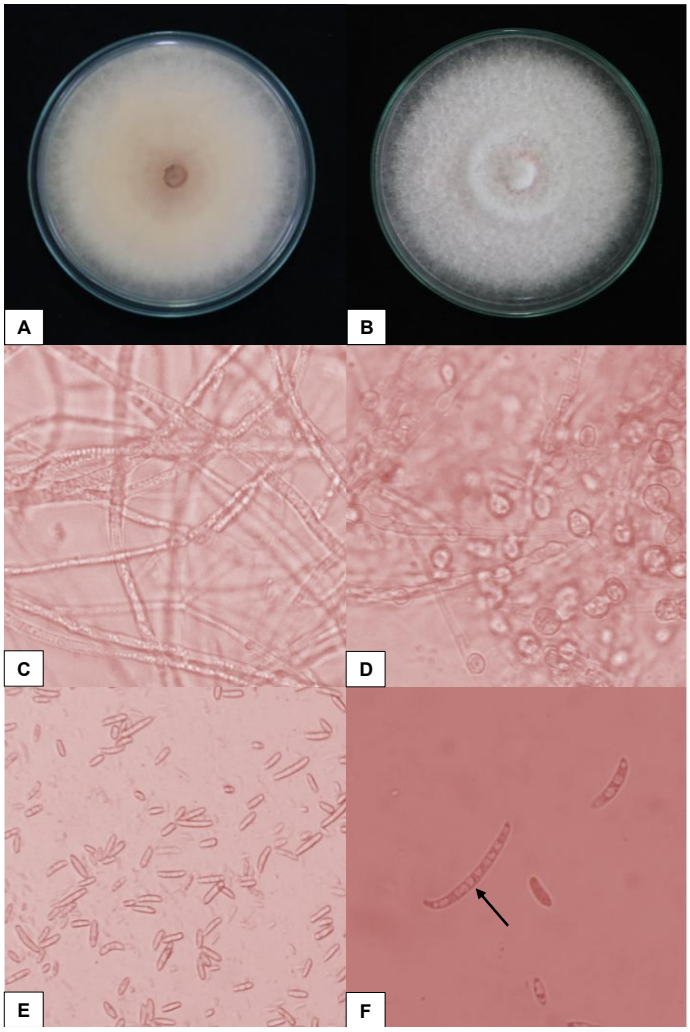

Figure 2. *(A-B) One-Month-Old Pure Culture of FocTR4 on the PDA Medium; (C) Mycelial Structure (40x); (D) Chlamydospores (40x); (E) Microconidia (40x); and (F) Macroconidia-Arrow (40x).*

Pathogenicity Testing of *Foc*TR4

A seven-day-old *Foc*TR4 pure culture was used for the pathogenicity testing. The fungus was mass-produced for inoculation in a 1:10 corn grits-to-sand substrate ratio. It was inoculated to a healthy Lakatan and Cavendish cv. Gran Naine banana seedlings to ensure the virulence of the fungus in different varieties. One hundred grams of *Foc*TR4 corn grits-to-sand substrate was used per pot, following the method of Chen et al. (2019) with slight modifications. The banana plants were gently removed from the pots and 50g of the substrate was added to the bottom of the pots before transplanting. Another 50g of the substrate was then spread evenly on the surface soil layer and covered with newspaper to maintain the soil moisture. Fourteen and 21 days after inoculation (DAI), the Lakatan and Gran Naine banana plants showed the symptoms of the disease, respectively, and these were isolated again using a half-strength PDA medium. A microscopic examination was done to confirm the identity of the fungus. The

infected plants were subjected to the histopathology study of the fungus intact to the tissue.

Histopathological Study of *Foc*TR4-infected Banana Varieties

The histopathological characterization was conducted following the method of Ara et al. (2017) with slight modification. Collection of specimens was done to the previously conducted pathogenicity testing of *Foc*TR4 isolates on the banana seedlings at 30DAI, showing the typical symptoms of Fusarium wilt. The roots and pseudostem strands of Lakatan and Cavendish cv. Gran Naine bananas from both healthy and *Foc*TR4-infected samples were collected and washed to undergo histopathology characterization. The collected roots and pseudostem strand underwent sectioning using a hand microtome to obtain thinned slices measuring 1-2mm, facilitating documentation of the fungal development on the tissue. Staining techniques were also employed using lactophenol-acid fuchsin to elucidate the intact structures of the pathogen within the plant host tissue.

Ethical Considerations

The study was conducted with strict adherence to ethical guidelines in the laboratory and nursery, ensuring the integrity of the research and the welfare of all involved. The research protocol received approval from the adviser. All banana varieties used were ethically sourced with proper permissions. The handling of *Foc*TR4 was performed in controlled conditions to avoid unintentional spread. Data collected were handled with utmost confidentiality and integrity, accessible only to authorized personnel. Safety protocols were rigorously followed to protect researchers and the environment, including the proper disposal of biological waste. This is important in ensuring the research contributed valuable scientific knowledge while upholding the highest ethical standards.

Results and Discussion

Pathogenicity Test

The present study evaluated the virulence of the *Fusarium oxysporum* f. sp. *cubense* TR4 isolates from the infected banana obtained from New Corella, Davao del Norte (Figure 1. A-C). The appearance of yellowing on the older leaves in both varieties (Figure 3. A-B) and the pseudostem splitting observed in the Gran Naine variety (Figure 3. B-red circle) are the common external symptoms of *Foc*TR4, indicating a pathogenic effect of the isolates to the banana seedlings. The initial symptoms of yellowing were observed early at 14DAI for Lakatan and 21DAI for the Gran Naine variety and splitting was observed at 30DAI. According to Vicente et al. (2014), the incubation period or the period from inoculation to the first symptoms observation might be variable depending on inoculation procedures, banana genotypes used, the aggressiveness of the *Foc*TR4 isolate, or due to an environmental condition.

The external symptoms observed were evident on the internal infection of *Foc*TR4 on the banana plants, showing the corm and vascular discoloration in both varieties (Figure 3. D and G) as compared to the observation of corm and vascular of healthy bananas (Figure 3. C and F). Browning of roots is also apparent on the *Foc*TR4-infected (Figure 3. E and H-right side), demonstrating a virulence of the *Foc*TR4 isolates. Thus, when the fungus penetrates, it remains within the xylem producing microconidia and toxins that move upstream in the plant sap, colonizing neighboring vessels and

producing a new fungal structure (Vicente et al., 2014); this causes the banana plant parts to become discolored.

In the study of Ploetz (2006), a strain of *Foc* causes a typical wilt withers condition on the infected banana plants joined by the necrosis and decaying of roots, rhizome, and pseudostem vessels. The most regular side effects become noticeable in susceptible banana plants after the presence of starting outer indications like light green streaks on the foundation of the petiole and the brown-ruddy staining of the vessels under the epidermis of the petiole. These manifestations happen somewhere in the range of 2-5 months after the disease of roots. Pegg et al. (2019) indicated the association of toxic metabolites, and fusaric acid produced by *Foc* and other *Fusarium* species is considered to contribute to the symptom expression. The banana plant might be delayed in showing external symptoms since it has a vascular limit of a few times its requirement for growth and reproduction.

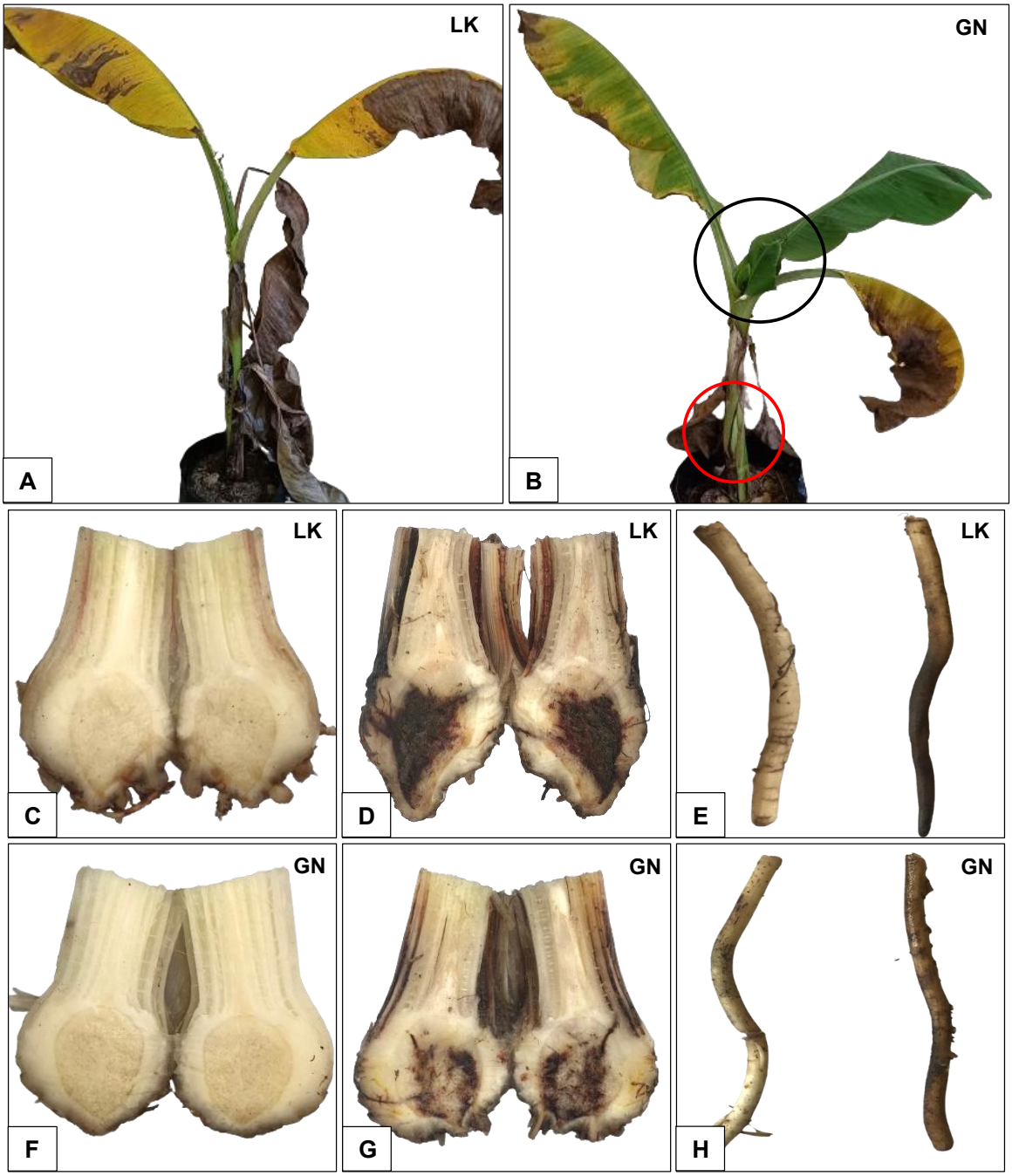

Figure 3. *Banana Seedlings at 30DAI. (A-B) FocTR4-Infected Showing Symptoms of Yellowing on the Older Leaves, Leaf Stunted, Rosetting-Black Circle, and Pseudostem Splitting-Red Circle; (C and F) Healthy Corm and Vascular of Banana; (D and G) FocTR4-Infected Corm and Vascular Discoloration of Banana; and (E and H) Healthy Roots (left side) and Infected Roots (right side).* ***LK*** *– Lakatan,* ***GN*** *– Gran Naine.*

Histopathological Profiling

The conducted pathogenicity testing of *Foc*TR4 on the banana seedlings at 30DAI, showing the typical symptoms of Fusarium wilt, external and internal (Figure 3. A-B), illustrated the entry of the pathogen and producing a fungal structure that elucidated in the histological activity. The cross-section of the roots (Figures 4 and 5. A), and pseudostem (Figure 6. A-B) of both Lakatan and Gran Naine banana varieties indicated a healthy structure of the cortex, parenchymatous cells (pith), xylem and phloem vessels, respectively.

The present study focused on the xylem vessels of the roots and vascular tissue of the pseudostem, in which the fungal structure was evident. The massive fungal mycelia were observed on the Lakatan variety, blocking the xylem cavities of the roots (Figure 4. C and D-my), considering the *Foc*TR4 symptoms on the pathogenicity observed early at 14DAI, compared to the Gran Naine variety with observed symptoms at 21DAI (Figure 3. E and H-right side). The massive colonization of fungal mycelia and microconidia was noticeable on the vascular tissue of the Lakatan pseudostem (Figure 6. C-my, D-micro). Also the *Foc*TR4-infected Gran Naine variety was able to produce a fungal structure in the vascular tissue (Figure 6. F-my, G-micro). Moreover, the *Foc*TR4 was able to produce a resting spore, the chlamydospore structure on the root xylem of both varieties (Figure 4 and 5. C and D-cs). Likewise, both varieties prominently exhibited a large yellow to brown discoloration on the cross-sectioned roots (Figures 4 and 5. C-dc) and pseudostem (Figure 6. C-D-dc, and F-G-dc).

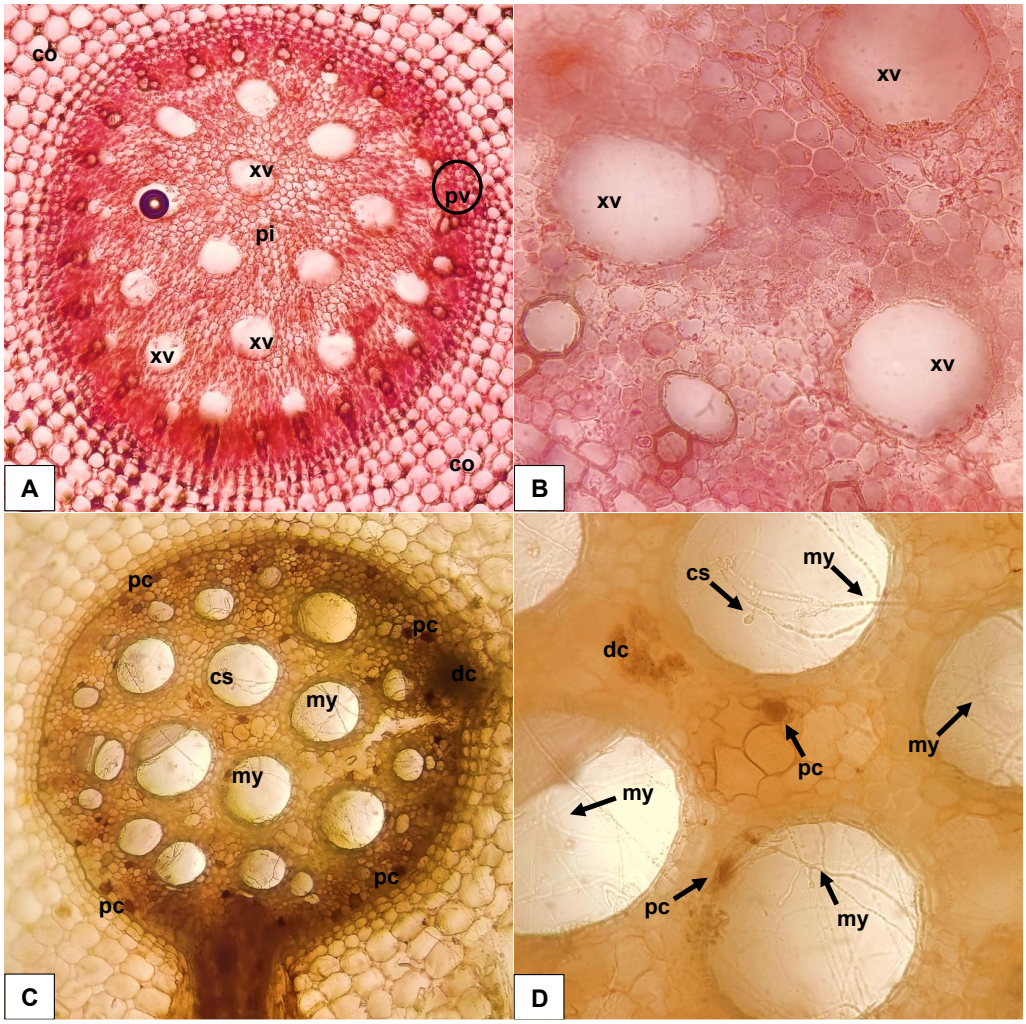

Figure 6. *Cross Section of the Roots of Lakatan Banana Seedlings at 30DAI. (A) Healthy Stele of the Banana Root (10x); (B) Close-Up of Xylem Vessels (40x); (C) FocTR4-Infected Stele of the Banana Root (10x); and (D) Close-Up of Xylem Vessels Infected with FocTR4 (40x).* ***co*** *– cortex,* ***pi*** *– pith,* ***xv*** *– xylem vessels,* ***pv*** *– phloem vessels-encircled,* ***pc*** *– phenolic compounds,* ***dc*** *– discoloration,* ***my*** *– mycelia,* ***cs*** *– chlamydospore.*

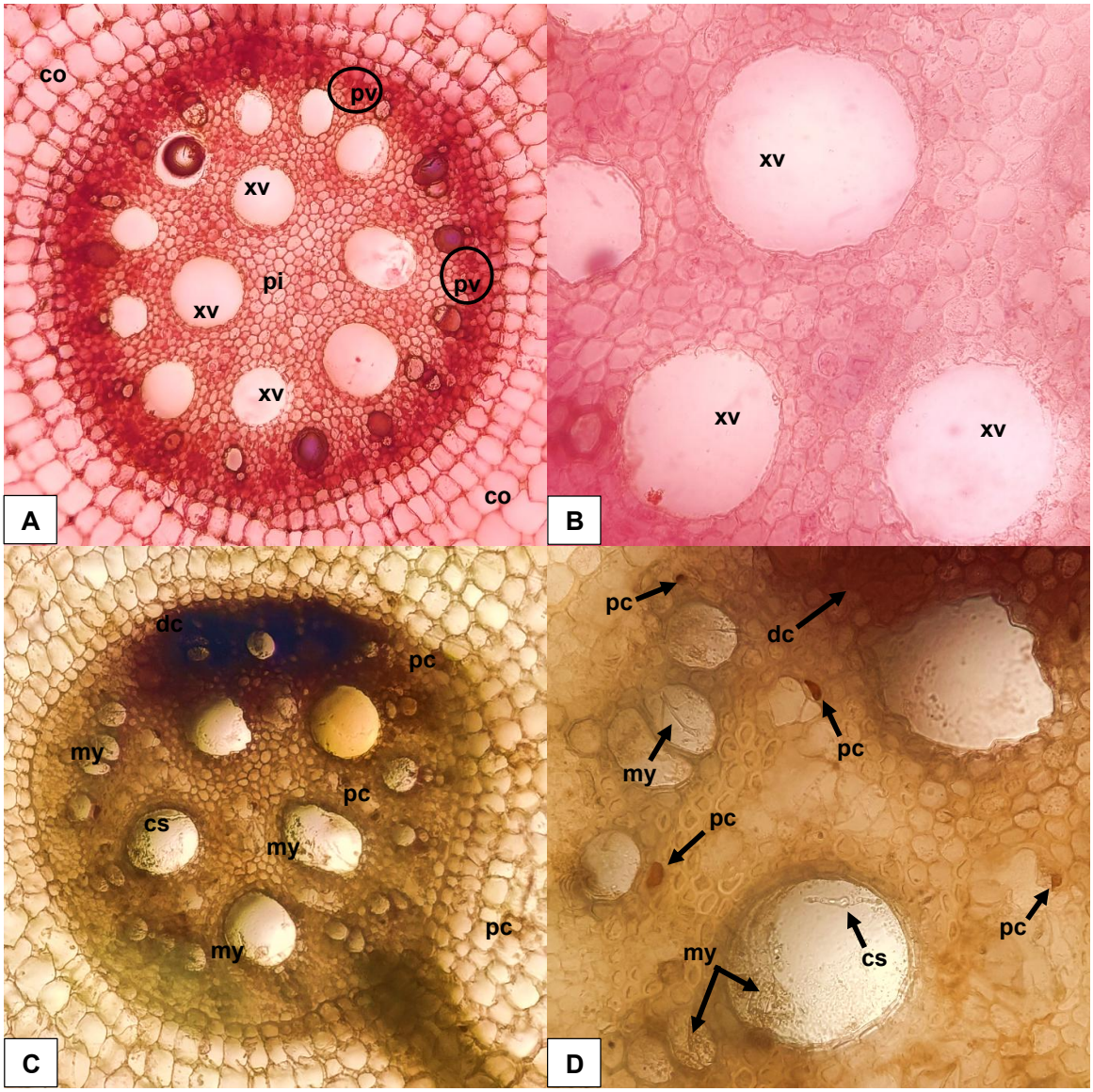

Figure 7. *Cross Section of the Roots of Gran Naine Banana Seedlings at 30DAI. (A) Healthy Stele of the Banana Root (10x); (B) Close-Up of Xylem Vessels (40x); (C) FocTR4-Infected Stele of the Banana Root (10x); and (D) Close-Up of Xylem Vessels Infected with FocTR4 (40x).* ***co*** *– cortex,* ***pi*** *– pith,* ***xv*** *– xylem vessels,* ***pv*** *– phloem vessels-encircled,* ***pc*** *– phenolic compounds,* ***dc*** *– discoloration,* ***my*** *– mycelia,* ***cs*** *– chlamydospore.*

The findings of the study coincide with Vishwanath et al. (2011), who demonstrated the invasion of pathogen at the root system and progressed to the rhizome part and pseudostem, causing a discoloration. During 30DAI, the presence of the fungal mycelia was observed from the root infected, and some parts of the xylem tissue showed a noticeable change, histologically. At 45DAI, an abundant fungal mycelium to the roots was prominent and a formation of the chlamydospores was also evident in a horizontal section of the roots.

Furthermore, the production of phenolic compounds by the plants was also elucidated in the *Foc*TR4-infected banana plants (Figure 4 and 5. C-pc), this can be produced as a defense mechanism in response to a pathogen attack. The observation was evident in both varieties; however, when considering the Lakatan variety, which demonstrated a higher level of *Foc*TR4 symptoms, the production of phenolic compounds by the plants was more pronounced and distinct compared to the Gran Naine variety. Thakker et al. (2013), indicated a proposition that the presence of such phenolic compounds implies the responsiveness of the plants to the pathogen, producing a mechanism through the building of its physical barriers.

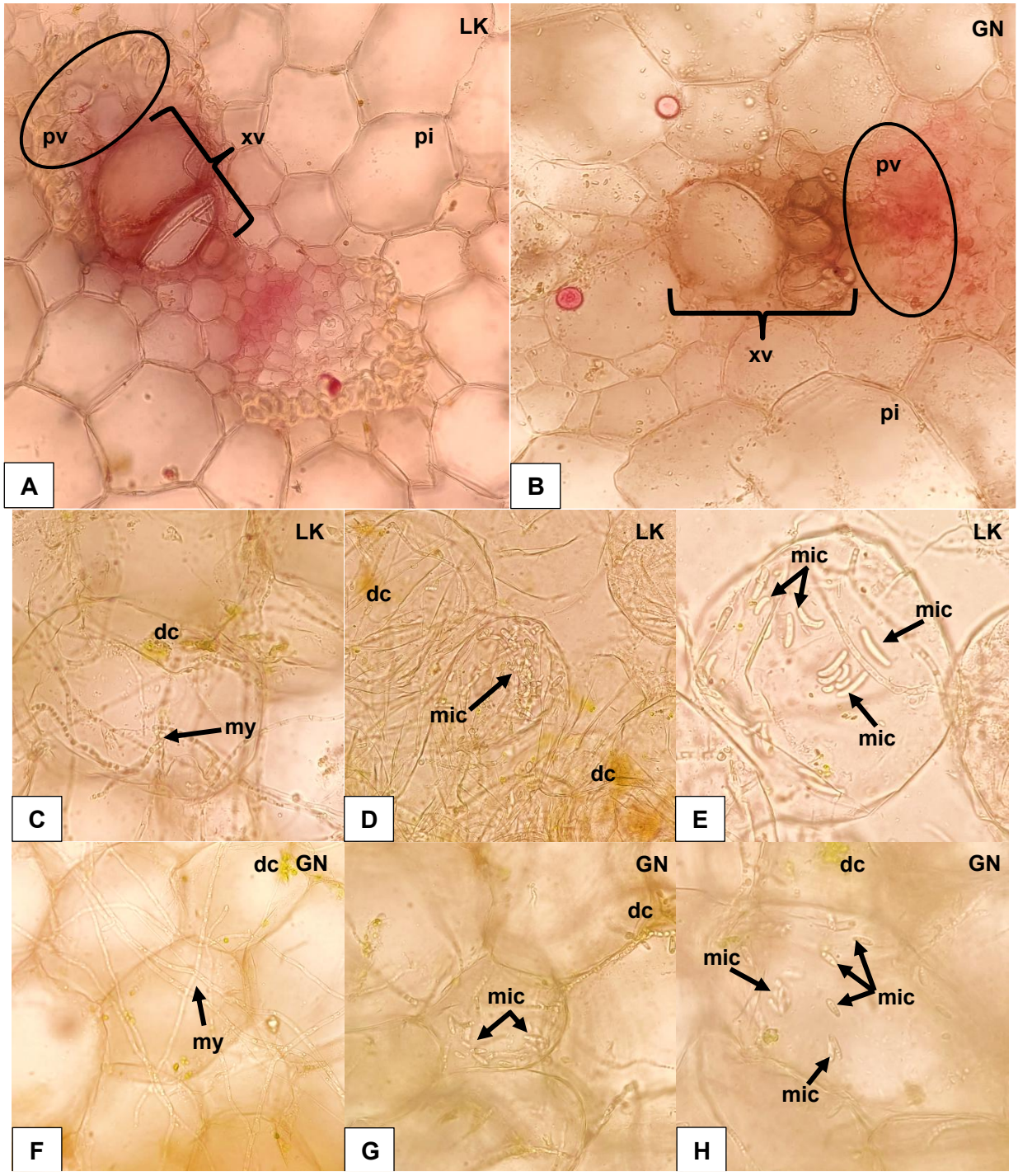

Figure 8. *Cross Section of the Pseudostem of Banana Seedlings at 30DAI. (A-B) Healthy Vascular Tissue of the Banana (40x); (C and F) FocTR4 Mycelial Structure Present in the Vascular Tissue-arrows (40x); (D and G) Massive Colonization of FocTR4 Microconidia in the Vascular Tissue-arrows (40x); and (E and H) Close-Up of Micronidia-arrows (40x).* ***LK*** *– Lakatan,* ***GN*** *– Gran Naine,* ***pi*** *– pith,* ***xv*** *– xylem vessels-arrows,* ***pv*** *– phloem vessels-encircled,* ***dc*** *– discoloration,* ***my*** *– mycelia,* ***mic*** *– microconidia.*

Conclusion and Future Works

The histopathological profiling of *Foc*TR4-infected banana seedlings in different varieties indicates substantial knowledge and information on the virulence and progression of the pathogen within the host plants. It enhances the understanding of host-pathogen interactions in Fusarium wilt disease. The present study demonstrated that the Lakatan variety exhibited earlier and more severe symptoms at 14DAI and extensive mycelial colonization of xylem vessels by 30DAI as compared to the Gran Naine variety. Both varieties showed characteristic internal and external symptoms, such as vascular discoloration, root browning, and the presence of fungal structures, including microconidia and chlamydospores. The production of such phenolic compounds was assumed to be observed on the stele of the roots of both varieties, as a defense response to pathogen attacks, which is more evident in the Lakatan variety.

These findings of the study suggest the aggressiveness of *Foc*TR4 and its capacity to rapidly infect and damage the banana plants. Thus, it is recommended to conduct further research, specifically on the molecular level, to understand the interaction of the banana plants and the pathogen. Also, exploring integrated management strategies to enhance the histological characteristics of *Foc*TR4-infected banana varieties, which includes the monitoring and early detection through different observation periods at the nursery and field condition, is also suggested.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. The biological materials used in this study, as well as all interactions with the school and company involved, were conducted transparently.