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EPIDEMIOLOGY AND YIELD LOSS ASSESSMENT OF BANANA FUSARIUM WILT (TROPICAL RACE 4) IN SINDH, PAKISTAN

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ABSTRACT

Panama disease, caused by *Fusarium oxysporum* f. sp. cubense (*Foc*), is a globally significant soil-borne disease threatening banana production. In Pakistan, the disease was first reported in 2014–15 and has since emerged as a major constraint to banana cultivation, particularly in Sindh province. This study aimed to assess the current distribution and severity of Panama disease, estimate associated yield losses, and characterize the *Foc* isolates responsible for the outbreak. Field surveys were conducted across eleven major banana-growing districts in Sindh, where disease incidence was recorded based on symptomatic assessment of plants in selected orchards. Infected samples were collected and subjected to both morphological and molecular identification using race- and lineage-specific primers. The average disease incidence across surveyed areas was 17%, with a maximum of 42% recorded in Thatta district. Based on these incidence rates and national production data, yield losses attributable to Panama disease were estimated at approximately 44,150 tonnes, indicating a 20% reduction in total banana output for 2022. Morphological analysis confirmed the presence of *Fusarium oxysporum*, and molecular diagnostics identified 15 out of 17 isolates as Tropical Race 4 (TR4). Two isolates, however, tested negative for both Race 4 and TR4-specific primers, suggesting the possible presence of other *Foc* races or novel variants. The widespread use of infected suckers, flood irrigation, and poor field sanitation were identified as key factors contributing to disease dissemination. These findings underscore the urgent need for containment strategies, including resistant cultivar development, improved diagnostic surveillance, and stricter quarantine enforcement. This study provides the first comprehensive overview of the Panama disease epidemic in Pakistan and highlights the threat posed by TR4 and potentially emerging *Foc* variants to national banana production and food security.

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INTRODUCTION

Banana (*Musa* spp.) is among the top ten agricultural commodities globally in terms of cultivated area, harvest yield, and caloric contribution, making it a staple food crop in many developing countries (Calberto et al., 2015). It is cultivated in more than 150 countries in tropical and subtropical regions and plays a pivotal role in the GDP of several developing countries. According to the Food and Agriculture Organization (FAO), bananas rank among the leading crops in terms of agricultural production and trade.

Production estimates indicate that global banana production has nearly doubled, rising from 66 million tonnes in 2000 to 135 million tonnes in 2022. The area under cultivation also increased from 4 million hectares in 2000 to about 6 million hectares in 2022. India, China, Indonesia, Nigeria, and Brazil are the top five banana-producing countries, with production figures of 35, 12, 9, 8, and 7 million tonnes, respectively. In contrast, Pakistan produced 0.2 million tonnes from an area of 33 thousand hectares in 2022 (FAOSTAT, 2023). In Pakistan, the majority of banana production (95%) occurs in Sindh province, which covers 33 thousand hectares, with 31 thousand hectares located in Sindh (PAKSTAT, 2023). The main districts for banana production are Thatta, Hyderabad, Badin, Mirpurkhas, Tando Allahyar, Matiari, Tando Muhammad Khan, Sanghar, Naushahro Feroze, Shaheed Benazirabad, and Khairpur. The primary variety grown in Pakistan is Basrai (Cavendish dwarf), which accounts for roughly 95% of production, with the remaining 5% being William Hybrid.

The banana crop is susceptible to various plant diseases, particularly those caused by fungi, which can result in significant production losses in terms of both fruit quality and quantity. These diseases include black sigatoka (*Pseudocercospora fijiensis* Morelet), yellow sigatoka (*Pseudocercospora musicola* Leach), and Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*, *Foc*), formerly known as Panama wilt (Alakonya et al., 2018; de Carvalho Santos et al., 2019; Ploetz, 2015a). Among banana diseases, Panama disease poses the greatest threat to banana production. The first documented description of Panama disease was reported in Australian bananas when Dr. Joseph Bancroft observed fungal wilt disease affecting banana plants ('Sugar,' AAB, Silk subgroup) at Eagle Farm, Brisbane, Australia, in 1874 (Bancroft, 1876). Another noteworthy report of Fusarium wilt appeared in Central America in 1890

(Ashby, 1913). The disease progressed as a slow-developing epidemic, spreading extensively. By the mid-twentieth century, it had decimated the Gros Michel banana industry in Central America.

The damage caused by *Foc* race 1 was alleviated by the shift to resistant Cavendish cultivars, which are the source of 99 percent of recent banana exports. Unfortunately, a new strain of *Foc* called Tropical Race 4 (TR4) was diagnosed for the first time in Taiwan, China, in 1967 (Su et al., 1986). TR4 infects not only Cavendish bananas but also many other banana varieties (Dita et al., 2018). Since then, TR4 has spread to many countries, resulting in substantial losses to banana production. In 2015, it was reported that *Foc* race 4 affects 40% of total Cavendish banana cultivation (Ploetz, 2015b). Besides targeting Cavendish bananas, this race also infects various other banana varieties, posing a significant threat to food security in regions where bananas serve as a staple food crop (Butler, 2013; Chen et al., 2013). Annual losses caused by this disease range from 60% to 90% in banana cultivation worldwide (Niwas et al., 2021).

Banana crop plantations, particularly in Pakistan, are at high risk due to the presence of Panama disease. This disease has the potential to cause extensive crop losses, comparable to the devastation caused by the banana bunchy top virus epidemic in the 1980s in Sindh, which nearly eradicated banana plantations in the province (Khalid et al., 1993). *Foc* exhibits two highly destructive races, Race 1 (R1) and Race 4 (R4), affecting the commercially important Cavendish group of bananas in India (Thangavelu et al., 2022). In Pakistan, however, only *Foc* TR4 has been reported, impacting the Cavendish variety in numerous areas in the Sindh province to date.

Preventing the further spread of this devastating banana disease has become imperative, primarily due to the lack of effective management options and the absence of resistant banana cultivars with market acceptability. The implementation and utilization of containment and quarantine strategies rely heavily on the early detection of the pathogen, involving a reliable diagnostic method (Carvalho et al., 2019). Furthermore, proper monitoring and identification of the physiological races of *Foc* are crucial for the avoidance and management of Fusarium wilt in bananas (Wu et al., 2019).

The rapid and widespread occurrence of Panama wilt disease across various cultivating regions in Sindh

province is alarming. Urgent measures are required to manage this disease, as its uncontrolled spread has the potential to devastate banana crops and associated industries in Pakistan (Muhammad et al., 2017). Given the significance and potential losses associated with this devastating banana disease, the present research work was undertaken to map the distribution of Panama disease of banana in Sindh province and to determine the *Foc* race(s) associated with it.

MATERIALS AND METHODS

Distribution and incidence of Panama disease

To determine the distribution and incidence of Panama disease, surveys were conducted in major banana-growing districts of Sindh province, namely Hyderabad, Tando Allahyar, Mirpurkhas, Sanghar, Matiari, Shaheed Benazirabad, Naushahro Feroze, Khairpur, Tando Mohammad Khan, Badin, and Thatta (Figure 1).

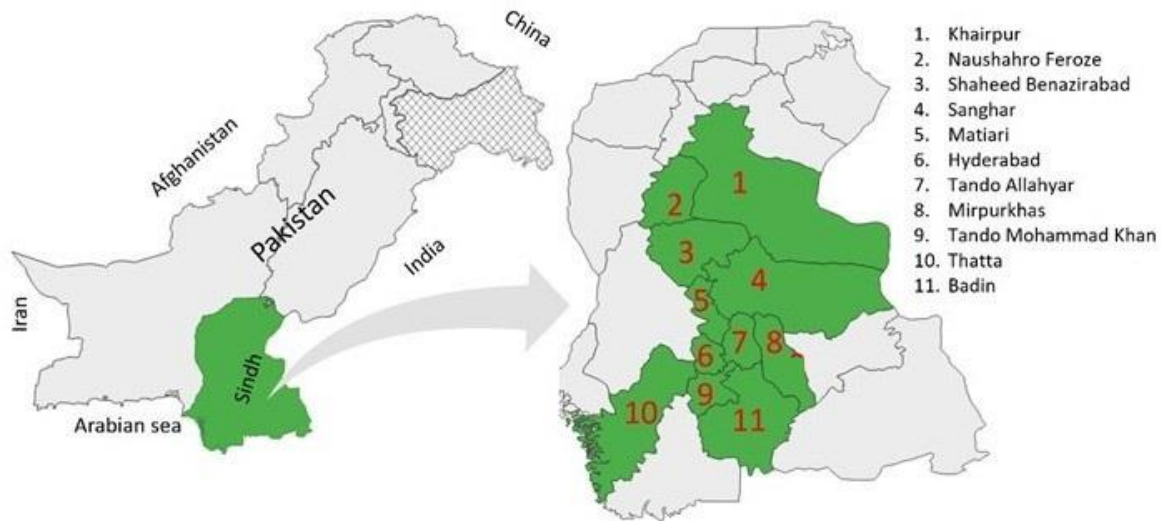


Figure 1. Map of Sindh, Pakistan showing sample collection districts.

From each district, five localities were randomly selected, and five banana orchards were visited within each locality, focusing on a one-acre area of crop per orchard. Banana plants showing typical wilt symptoms were examined for both external and internal indicators. Externally, affected plants exhibited chlorosis beginning with older leaves and progressing towards younger ones. Internally, brown discoloration of the rhizome and pseudostem was observed. Disease incidence was estimated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of wilted banana plants}}{\text{Total number of plants studied}} \times 100$$

During the survey, factors that may contribute to disease spread were recorded through photographs and personal discussions with farmers. The outcomes of these observations are presented in the results section.

Yield Loss

The average disease incidence was calculated using data from all surveyed districts, resulting in an overall

incidence rate of 17%. Based on Pakistan's banana production data for 2022 (FAOSTAT, 2023), yield loss due to the disease was estimated by calculating the difference between the total yield in the absence of disease and the actual yield obtained. The following formula was used:

$$\text{Yield loss} = \text{Total yield} - \text{Actual yield}$$

Sample Preparation

Samples were collected from diseased sections of the pseudostem and rhizome of banana plants showing typical Panama wilt symptoms. These samples were carefully stored in paper bags and transported to the laboratory. The tissues were then dissected into small sections containing discolored vascular strands, blotted dry using blotting paper, and preserved for subsequent fungal isolation.

Isolation and Morphological Identification of Isolates:

Isolation was carried out from the pseudostem or rhizome sections of wilted banana plants, particularly

from areas exhibiting typical continuous vascular discoloration. Small tissue segments, approximately 3 to 6 mm in length, were excised after surface sterilization with commercial bleach. These sections were then placed on half-strength potato dextrose agar (PDA) medium, supplemented with an antibacterial agent such as streptomycin solution (1g of streptomycin sulfate powder to 100 mL distilled water) at a concentration of 1.2 mL per 240 mL of PDA (Ainsworth, 1971). Emerging colonies were re-isolated to prepare monoconidial cultures. Identification was performed based on the morphological and cultural characteristics of the fungi,

following the guidelines of Leslie and Summerell (2006).

Molecular Identification of Isolates

DNA was extracted using the CTAB method from all *Fusarium* isolates grown on PDA (Oxoid) at 25 °C for 7 days (Manickkam and Moses, 2023). PCR-based molecular diagnostics were carried out using the primer set *SIX9_Foc_F/SIX9_Foc_R* (Carvalhais et al., 2019) to identify *Foc* isolates. *Foc* Race 4-specific primers *Foc-1/Foc-2* (Lin et al., 2009) were employed to detect *Foc* Race 4, while *Foc* TR4-specific primers *FocTR4-F/FocTR4-R* (Dita et al., 2010) were used for TR4 identification (Table 1).

Table 1. List of primers used in this study.

Primer Name	Base Sequence (5'→3')	Annealing Temp (°C)	Fragment size (bp)	Specificity	Reference
<i>SIX9_Foc_F/SIX9_Foc_R</i>	<u>ATCGCTGAAGCCCAGAACAA</u> <u>TTCTGTCCGTCGATCGTTCC</u>	58	260	All <i>Foc</i>	Carvalhais <i>et al.</i> (2019)
<i>Foc-1/Foc-2</i>	<u>CAGGGGATGTATGAGGAGGCT</u> <u>GTGACAGCGTCGTCTAGTTCC</u>	53	242	<i>Foc</i> R4	Lin <i>et al.</i> (2009)
<i>FocTR4-F/FocTR4-R</i>	<u>CACGTTTAAGGTGCCATGAGAG</u> <u>CGCACGCCAGGACTGCCTCGTGA</u>	53	463	<i>Foc</i> TR4	Dita <i>et al.</i> (2010)

All PCR amplifications were performed in 25 µL reaction volumes using Biobasic Taq PCR master mix, following the manufacturer's instructions. The final concentrations of DNA template and primers in each reaction were 5 pg/µL and 0.4 µM, respectively. The PCR cycling conditions included an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 60 seconds, annealing at primer-specific temperatures for 30 seconds, and extension at 72 °C for 60 seconds. A final extension was carried out at 72 °C for 10 minutes.

Agarose gel electrophoresis was used to visualize PCR products. Samples, along with a 100 bp DNA ladder (Thermo Scientific), were loaded on a 1.5% (w/v) agarose gel prepared in 1X TAE buffer containing 0.5 µg/mL ethidium bromide. Electrophoresis was conducted at 90 volts for 2 hours, and the bands were visualized using a gel documentation system.

RESULTS

Symptoms of Panama Disease

Diseased plants were identified based on characteristic symptoms visible even from a distance, primarily including leaf chlorosis and wilting. Affected plants

displayed progressive chlorosis starting from older leaves and extending to younger ones. Chlorosis typically began at the leaf margins and progressed inward, eventually turning the entire leaf yellow. This was followed by complete tissue necrosis, with the leaves bending at the petiole and hanging around the pseudostem (Figure 2a). As the disease progressed, the younger leaves also exhibited similar symptoms and began to droop.

Cross-sectional examination of the pseudostem revealed the presence of clogging substances and dark brown discoloration (Figure 2b & 2c). Reddish to brown discoloration of vascular tissues was observed, particularly in the rhizome around the stele corners (Figure 2d). Rotting and discoloration of feeder roots were also evident.

Distribution and Incidence of Panama Disease

The percentage of Panama disease-infected plants was recorded across banana-growing districts, including Hyderabad, Tando Allahyar, Mirpurkhas, Sanghar, Matiari, Shaheed Benazirabad, Naushahro Feroze, Khairpur, Tando Mohammad Khan, Badin and Thatta in Sindh province of Pakistan.

The occurrence of Panama disease was recorded in all surveyed districts, with incidence rates varying across locations. The average disease incidence ranged from 2% to 42%. The highest incidence was observed in Thatta

(42%), followed by Mirpurkhas (30%), Matiari (21%), and Shaheed Benazirabad (20%). The lowest incidence was recorded in Khairpur (2%), followed by Naushahro Feroze (7%) and Sanghar (10%) (Figure 3).

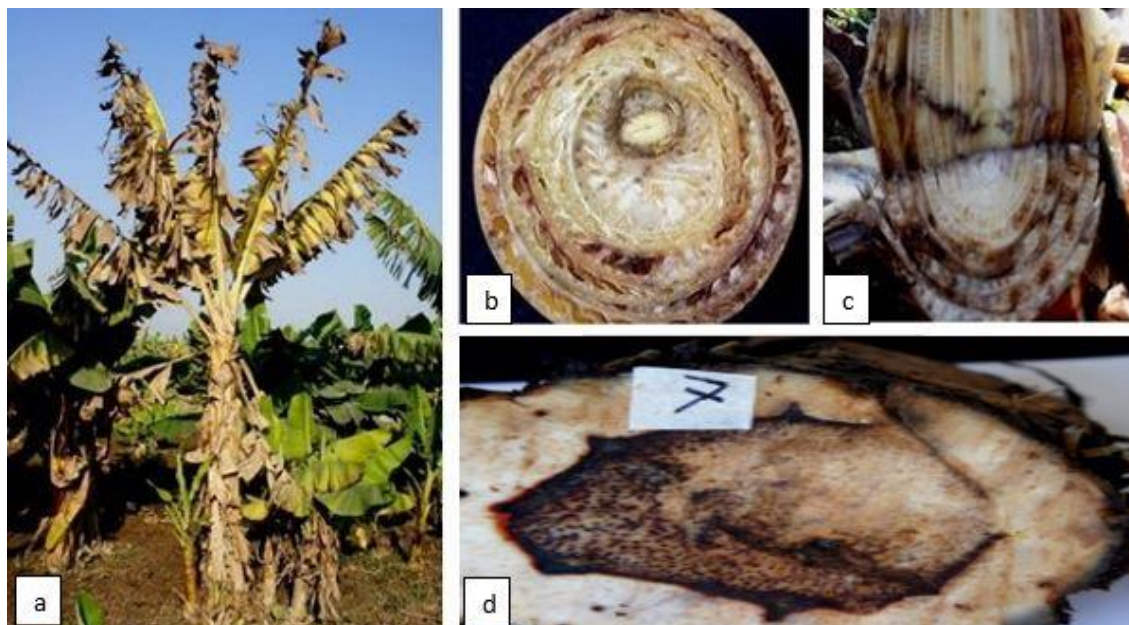


Figure 2. Symptomatology of Panama disease. (a) Leaf chlorosis and wilting of banana plant, (b & c) discoloration of pseudostem and (d) discoloration of rhizome.

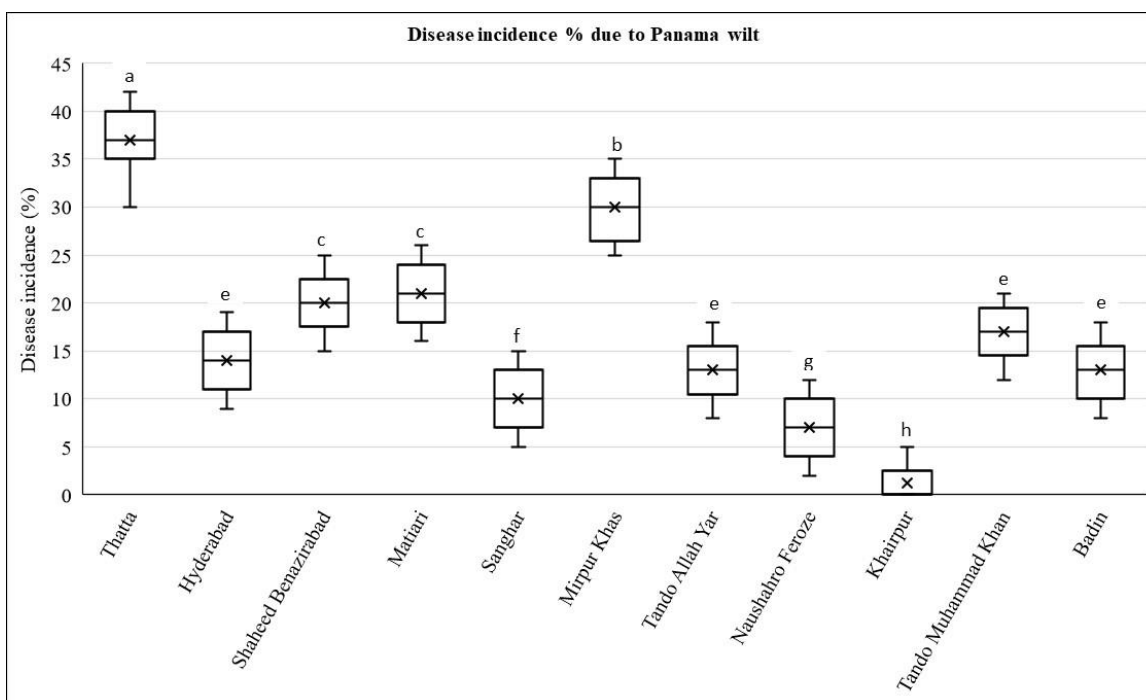


Figure 3. Disease incidence of Panama disease in Cavendish banana across different districts of Sindh, Pakistan. *Means followed by the same letters are not significantly different from each other (LSD = 1.969, $P < 0.05$).

Production Losses

Assuming that each infected plant results in complete wilt and a total loss of yield for that plant, the disease incidence percentage and yield loss percentage would be equal in this scenario. The average disease incidence, calculated by considering data from all surveyed districts, is found to be 17%. Utilizing Pakistan's banana production data from FAOSTAT (2023), which states a production of 215,570 tonnes, the yield loss can be calculated as follows:

Given the average incidence of 17%, it implies that 17% of the plants succumbed to the disease, leaving 83% of the plants surviving. These surviving plants produced a yield of 215,570 tonnes. Therefore, the equation can be expressed as $0.83 \times Y = 215,570$. Solving for Y provides us with the total yield that would have been obtained without any disease. The yield loss due to the disease is then the difference between the total yield without disease and the actual yield obtained. The total yield without any disease would be 259,720 tonnes ($Y = 215,570 / 0.83 \approx 259,720$). Therefore, the calculated yield loss is 44,150 tonnes (Yield loss = $Y - 215,570 = 259,720 - 215,570 = 44,150$). This indicates that a 17% increase in production could have been achieved in the absence of Panama disease (Figure 4).

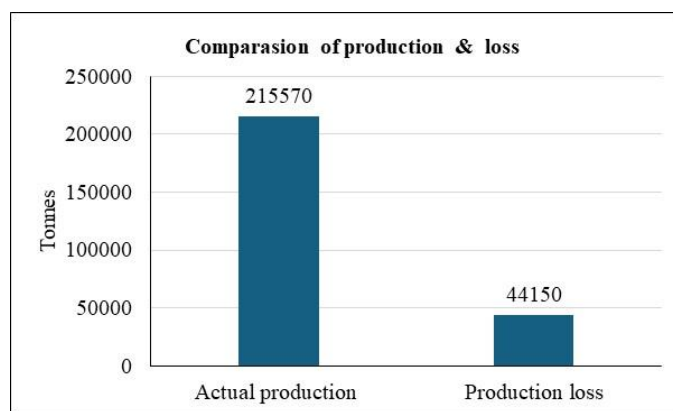


Figure 4. Comparison of banana production during 2022 and production loss due to Panama disease of banana.

Factors Contributing to Disease Spread and Loss

Panama disease of banana poses a significant threat to agricultural productivity, leading to substantial yield losses. This complex issue is not solely attributable to a single factor, but rather to a web of interconnected elements contributing to its severity.

During the survey, attempts were made to record such factors. One major contributor may be irrigation by flooding, which has facilitated the spread of the disease to a larger area. This, coupled with the widespread practice of establishing new plantations from suckers that may be infected yet symptomless, exacerbates the problem. Furthermore, other contributing factors include inappropriate water management practices, such as flood irrigation, which can lead to waterlogging and increased disease incidence. Improper field management, such as high planting density and inadequate nutrient management, further weakens crop resilience and attracts pests. Inadequate eradication and post-eradication treatments also play a role in perpetuating the issue (Figure 5).

Morphological identification of isolates

Isolation from disease samples collected from 11 districts of Sindh province in Pakistan revealed the association of *Foc* with wilted banana plants. In general, for all 17 isolates, we observed that they produced white to creamy aerial mycelium on PDA medium. All the isolates produced 3–5 septate, slightly sickle-shaped macroconidia with an attenuated apical cell along with a foot-shaped basal cell. The isolates also produced one- or two-celled microconidia that were either oval or kidney-shaped. Chlamydo spores were round and thick-walled, produced singly or in pairs. These characteristics are consistent with *F. oxysporum*, as described by Leslie and Summerell (2006).

Molecular identification of isolates

In our study, PCR-based molecular diagnostics revealed the presence of *Foc* (TR4) causing Panama disease in surveyed banana-growing areas of Sindh (Table 2). All 17 isolates were identified based on morphological and cultural characteristics as *F. oxysporum*, and were subjected to PCR-based molecular diagnostics using the primer set SIX9_ *Foc*_F/SIX9_ *Foc*_R. A 260 bp amplification product was obtained, which is the expected band size for all *Foc* isolates. Accordingly, all 17 isolates were identified as *Foc*. These were further subjected to PCR using *Foc* Race 4-specific primers (*Foc*-1/*Foc*-2), which yielded a 242 bp amplification product in 15 isolates, confirming their identity as *Foc* Race 4 (Table 2).

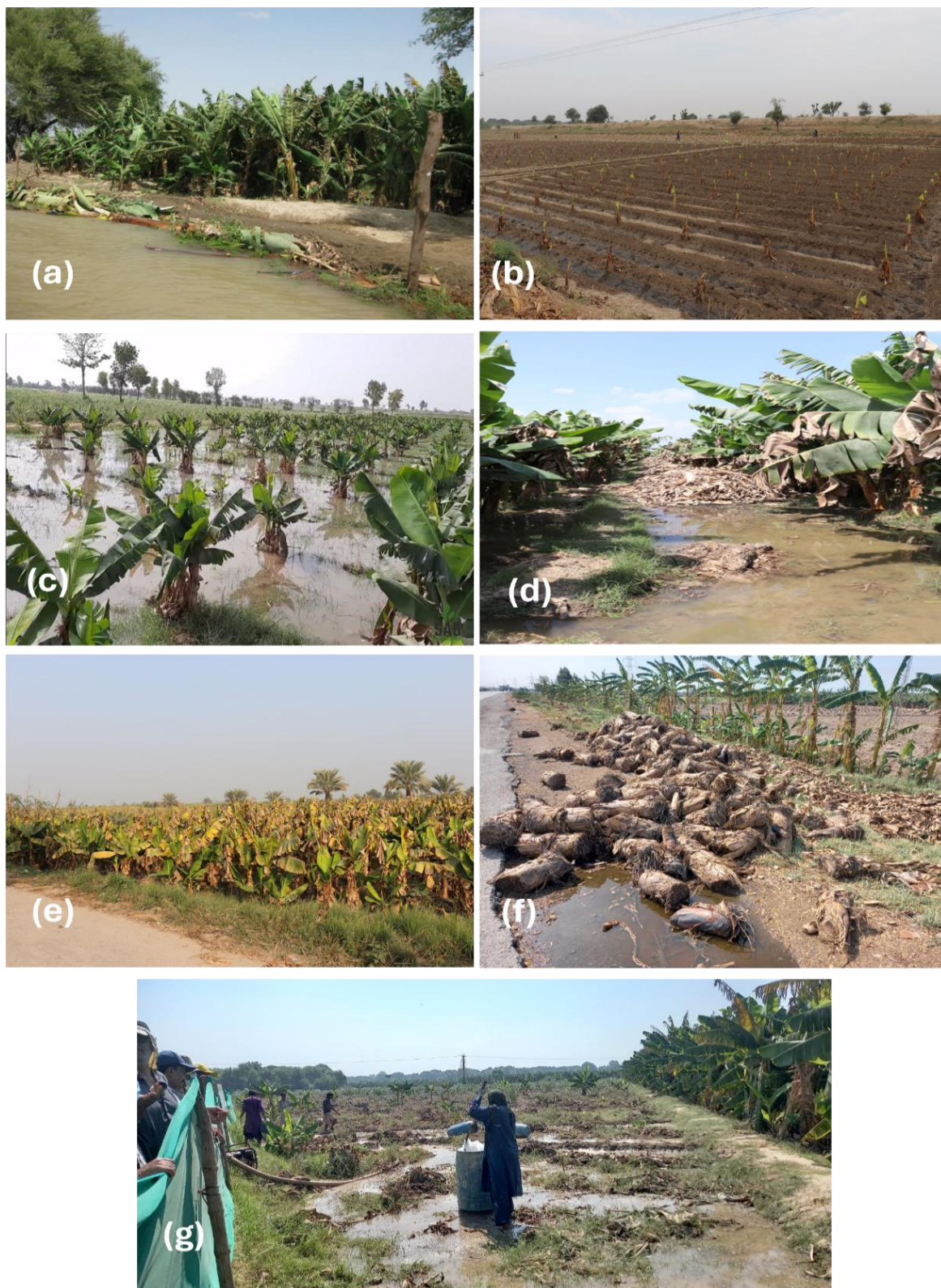


Figure 5. Cultural practices that spread and enhance Panama disease severity. (a) Flood; (b) Establishing new banana field from suckers of older plants (c) Flood irrigation in banana field; (d) Crop residues facilitation inoculum dispersal; (e) High density and poorly managed field; (f) Improper destruction of infected banana; and (g) Improper eradication of infected field.

Table 2. Response of all isolates obtained from different districts of Sindh to primer set for all *Foc* and specific primer set for Race 4 and TR4.

Sr. No	Name of District	Isolate code	Name of Primers		
			All <i>Foc</i>	Race 4	TR4 (VCG 01213)
			SIX9_ <i>Foc</i> _F/ SIX9_ <i>Foc</i> _R	<i>Foc</i> -1/ <i>Foc</i> -2	<i>Foc</i> TR4-F/ <i>Foc</i> TR4-R
1	Thatta	<i>Foc</i> -1	+	+	+
		<i>Foc</i> -2	+	+	+
		<i>Foc</i> -3	+	+	+
2	Hyderabad	<i>Foc</i> -4	+	+	+
		<i>Foc</i> -5	+	-	-
3	Shaheed Benazirabad	<i>Foc</i> -6	+	+	+
4	Matiari	<i>Foc</i> -7	+	+	+
5	Sanghar	<i>Foc</i> -8	+	+	+
6	Mirpur Khas	<i>Foc</i> -9	+	-	-
		<i>Foc</i> -10	+	+	+
7	Tando Allah Yar	<i>Foc</i> -11	+	+	+
8	Naushahro Feroze	<i>Foc</i> -12	+	+	+
9	Khairpur	<i>Foc</i> -13	+	+	+
10	Tando Muhammad Khan	<i>Foc</i> -14	+	+	+
		<i>Foc</i> -15	+	+	+
11	Badin	<i>Foc</i> -16	+	+	+
		<i>Foc</i> -17	+	+	+

In addition, we conducted PCR using *Foc* TR4-specific primers (*Foc*TR4-F/*Foc*TR4-R), and amplification products of 463 bp were obtained for all Race 4 *Foc* isolates, which is the expected band size for *Foc* TR4. The results of the present study revealed that 15 *Fusarium oxysporum* isolates were *Foc* TR4. Two *Foc* isolates, from Mirpurkhas and Hyderabad, were not identified using *Foc* Race 4 or *Foc* TR4-specific primers (Table 2).

DISCUSSION

The Panama disease of banana is threatening banana cultivation in major parts of the world, including Pakistan. Most bananas grown in Pakistan are of the Basrai (Dwarf Cavendish) variety, which is susceptible to *Foc* Race 4. Consequently, the present investigation was conducted to map the distribution of Panama disease in Sindh, Pakistan, and to determine the responsible *Foc* race(s). In the study area (Sindh, Pakistan), the above-ground parts of affected banana plants exhibited typical

chlorosis, yellowing, and wilting, while the pseudostem and rhizome showed characteristic internal brownish discoloration. Such symptoms have also been observed in other parts of the world (Moore et al., 1995; Su et al., 1986; Ploetz, 2015b). In the beginning, the infection starts with the roots, resulting in the production of gums, tyloses, and gel in the plant xylem lumen. While pathogen infections are primarily blocked, in rare instances they become systemic, passing through the pseudostem and rhizome (Ho et al., 2015; Li et al., 2011; Zhang et al., 2018). Colonization of the pathogen in the infected rhizome is considered the most proficient stage (Li et al., 2017).

In our current survey, the disease was recorded in all major banana-growing districts with varying incidence. The average disease incidence ranged from 2% to 42%. Panama disease was first reported in Pakistan by Syed et al. (2015), and since then, it has spread widely. Over time, and due to the lack of an effective management

strategy, it has become prevalent in many other areas of Sindh province. The primary reasons for its rapid spread include the absence of strict domestic quarantine measures, coupled with poor on-farm practices such as flood irrigation, propagation through infected suckers, and inadequate disposal of plant debris.

The present study also underscores the significant destructive potential of Panama disease. With an average disease incidence of only 17%, the estimated yield losses are still substantial amounting to approximately 20.5%. Panama disease remains one of the most damaging afflictions affecting banana crops worldwide (Rebouças et al., 2021). Since the 1980s, the spread of *Foc* (TR4) has led to severe infestations in Cavendish banana cultivation (Segura-Mena et al., 2021). Crop losses due to *Foc* directly affect the subsistence, income, and nutrition of thousands of people. In Vietnam, forecasts predict an 8% reduction in banana cultivation area within the next five years, and a potential increase to 71% within 25 years if effective management strategies are not adopted (Le Thi et al., 2022). Moreover, the distribution of *Foc* (TR4) is estimated to affect 17% of the world's banana cultivation area by 2040, resulting in a projected loss of 36 million tonnes of bananas, valued at more than 10 billion US dollars (Staver et al., 2020).

The genetic variation in the Panama disease pathogen has resulted in the emergence of several *Foc* races. The present study clearly indicates that one or more races of *Foc* are distributed across banana-growing regions of Sindh, with TR4 being the most dominant. However, in two specific locations, the isolates tested positive for *Foc* but could not be identified as Race 4 or TR4 using race-specific primers. This suggests that at least 2 out of 17 isolates may belong to a race other than Race 4 and/or TR4. These could potentially be Race 1 or Race 2, or they may represent novel *Foc* variants. Based on pathogenicity to differential host cultivars, *Foc* is classified into three physiological races. *Foc* Race 1 (R1) affects 'Lady Finger' (AAB), 'Gros Michel' (AAA), and 'Pisang Awak' (ABB). *Foc* Race 2 (R2) affects 'Bluggoe' (ABB) and closely related ABB clones. *Foc* Race 4 (R4) affects most cultivars susceptible to R1 and R2, particularly the Cavendish subgroup (AAA) (Bai et al., 2020; Buddenhagen, 2009; Ploetz, 2015b; Siamak and Zheng, 2018). *Foc* R4 is further divided into Tropical Race 4 (TR4) and Subtropical Race 4 (SR4), depending on their pathogenicity in different climatic conditions (Ploetz and Pegg, 2000; Viljoen, 2002). TR4 infects

Cavendish bananas in both tropical and subtropical regions, whereas SR4 is limited to subtropical climates (Buddenhagen, 2009; Ploetz, 2006). Among all races, TR4 is considered the most critical due to its survivability, wide host range, and greater virulence (Thangavelu et al., 2020). Recently, the first occurrence of *Foc* Race 1 in the cultivar Grand Naine was reported from India (Thangavelu et al., 2021). *Foc* R1 and R4 are the two most predominant races affecting Cavendish bananas in India (Thangavelu et al., 2022). Therefore, it is imperative to identify these unidentified strains from Pakistan through further molecular and pathogenic characterization.

CONCLUSIONS

Foc (TR4) is the primary causal agent of Panama disease in bananas, and, due to the lack of effective management strategies, this lethal disease continues to threaten banana cultivation across the globe, including Pakistan. To address this issue at the national level, further research is required to develop containment and management strategies, including the use of plant resistance inducers. Such efforts are essential for mitigating the impact of the disease and safeguarding food security, as well as reducing economic losses to the banana industry.

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REFERENCES

- Ainsworth, G. C. 1971. Ainsworth and Bisby's Dictionary of the Fungi. 6th ed. Commonwealth Mycological Institute, Kew, Surrey, England. 663 pp.
- Alakonya, A. E., J. Kimunye, G. Mahuku, D. Amah, B. Uwimana, A. Brown, and R. Swennen. 2018. Progress in understanding Pseudocercospora banana pathogens and the development of resistant Musa germplasm. *Plant Pathology*, 67(4), 759-770.
- Ashby, S. F. 1913. Banana disease in Jamaica. *Science* 31: 754-755.
- Bai, T., M. Qin, X. Li, H. Fan, S. Xu, L. Zeng, and S. J. Zheng. 2020. An additional threat to 'Cavendish' banana growers and traders: The infection of banana peduncles by *Fusarium oxysporum* f. sp. *cubense*

- Tropical Race 4 (*Foc* TR4). *Plant Health Progress*, 21(4): 312-316.
- Bancroft, J. 1876. Report of the board appointed to enquire into the cause of disease affecting livestock and plants. *Votes Proc*, 3, 1011-1038.
- Buddenhagen, I. 2009. Understanding strain diversity in *Fusarium oxysporum* f. sp. *cubense* and history of introduction of "Tropical Race 4" to better manage banana production. *Acta Hort.* 828, 193-204. doi: 10.17660/ActaHortic.2009.828.19
- Butler, D. 2013. Fungus threatens top banana: fears rise for Latin American industry as devastating disease hits leading variety in Africa and Middle East. *Nature*, 504(7479), 195-197.
- Calberto, G., C. Staver, and P. Siles. 2015. An assessment of global banana production and suitability under climate change scenarios. In: *Climate Change and Food Systems: Global Food Assessments and Implications for Food Security and Trade*. E. Aziz, ed. Food and Agriculture Organization of the United Nations, Rome. Pages 264-291.
- Carvalho, L. C., J. Henderson, V. A. Rincon-Florez, C. O'Dwyer, E. Czulowski, E. A. Aitken, and A. Drenth. 2019. Molecular diagnostics of banana *Fusarium* wilt targeting Secreted-in-Xylem genes. *Frontiers in Plant Science*, 547.
- Chen, Q., B. Li, P. Liu, C. Lan, Z. Zhan, and Q. Weng. 2013. Development and evaluation of specific PCR and LAMP assays for the rapid detection of *Phytophthora melonis*. *European Journal of Plant Pathology*, 137(3), 597-607.
- de Carvalho Santos, T. T., V. B. de Oliveira Amorim, J. A. dos Santos-Serejo, C. A. da Silva Ledo, F. Haddad, C. F. Ferreira, and E. P. Amorim. 2019. Genetic variability among autotetraploid populations of banana plants derived from wild diploids through chromosome doubling using SSR and molecular markers based on retrotransposons. *Molecular Breeding*, 39(7), 1-13.
- Dita, M. A., C. Waalwijk, I. W. Buddenhagen, M. T. Souza Jr, and G. H. J. Kema. 2010. A molecular diagnostic for tropical race 4 of the banana *Fusarium* wilt pathogen. *Plant Pathology*, 59(2): 348-357.
- Dita, M., M. Barquero, D. Heck, E. S. Mizubuti, and C. P. Staver. 2018. *Fusarium* wilt of banana: current knowledge on epidemiology and research needs toward sustainable disease management. *Frontiers in plant science*, 9, 1468.
- FAOSTAT. 2023. <https://www.fao.org/faostat/en/#data/QCL>
- Ho, Y. N., H. M. Chiang, C. P. Chao, C. C. Su, H. F. Hsu, C. T. Guo, J. L. Hsieh and C. C. Huang. 2015. In planta biocontrol of soilborne *Fusarium* wilt of banana through a plant endophytic bacterium, *Burkholderia cenocepacia* 869T2. *Plant and Soil*, 387(1), 295-306.
- Khalid, S., M. H. Soomro, and R. H. Stover, 1993. First report of banana bunchy top virus in Pakistan. *Plant Disease*, 77(1).
- Le Thi, L., A. Mertens, D. T. Vu, T. D. Vu, P. L. A. Minh, H. N. Duc, S. D. Backer, R. Swennen, F. Vandeloock, B. Panis, M. Amalfi, C. Decock, S. I. F. Gomes, V. S. F. T. Merckx and S. B. Janssens. 2022. Diversity of *Fusarium* associated banana wilt in northern Viet Nam. *MycKeys*, 87, 53.
- Leslie, J. F., B. A. Summerell. 2006. *The Fusarium laboratory manual*. Ames: Blackwell Publishing. <https://doi.org/10.1002/9780470278376>.
- Li, C., S. Chen, C. Zuo, Q. Sun, Q. Ye, G. Yi, and B. Huang. 2011. The use of GFP-transformed isolates to study infection of banana with *Fusarium oxysporum* f. sp. *cubense* race 4. *European Journal of Plant Pathology*, 131(2), 327-340.
- Li, C., J. Yang, W. Li, J. Sun, and M. Peng. 2017. Direct root penetration and rhizome vascular colonization by *Fusarium oxysporum* f. sp. *cubense* are the key steps in the successful infection of Brazil Cavendish. *Plant Disease*, 101(12), 2073-2078.
- Lin, Y. H., J. Y. Chang, E. T. Liu, C. P. Chao, J. W. Huang, and P. F. L. Chang. 2009. Development of a molecular marker for specific detection of *Fusarium oxysporum* f. sp. *cubense* race 4. *European Journal of Plant Pathology*, 123(3): 353-365.
- Manickam, J., & M. J.P. 2023. Immunotoxicity of methanol extract of Entomopathogenic fungi, *Aspergillus niger* (OM514698) against House fly (*Musca domestica*) larva. <https://europepmc.org/article/ppr/ppr593008>.
- Muhammad, A., I. Hussain, K. A. Khanzada, L. Kumar, M. Ali, T. Yasmin, and M. Z. Hyder. 2017. Molecular characterization of *Fusarium oxysporum* f. sp. *Cubense* (*Foc*) tropical race 4 causing Panama disease in Cavendish banana in Pakistan. *Pakistan Journal of Agricultural Sciences*, 54(1).
- Moore, N. Y., S. Bentley, K. G. Pegg, and D. R. Jones. 1995. *Fusarium* wilt of banana. *Musa* disease fact sheet.

- Niwas, R., G. Chand, and R. N. Gupta. 2021. Fusarium Wilt: A Destructive Disease of Banana and Their Sustainable Management. In (Ed.), *Fusarium - An Overview of the Genus*. IntechOpen. <https://doi.org/10.5772/intechopen.101496>
- PAKSTAT. 2022. [Pakistan Statistical Year Book 2022.pdf](https://www.pbs.gov.pk/Pakistan%20Statistical%20Year%20Book%202022.pdf) (www.pbs.gov.pk)
- Ploetz, R., and K. Pegg. 2000. Fusarium wilt. In *Diseases of Banana, Abacá and Enset*, ed D. Jones (Wallingford: CABI Publishing), 143–159.
- Ploetz, R. C. 2006. Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp. *cubense*. *Phytopathology*, 96(6): 653-656.
- Ploetz, R. C. 2015a. Fusarium wilt of banana. *Phytopathology*, 105(12), 1512-1521.
- Ploetz, R. C. 2015b. Management of Fusarium wilt of banana: A review with special reference to tropical race 4. *Crop Protection*, 73: 7-15.
- Rebouças, T. A., A. de Jesus Rocha, T. S. Cerqueira, P. R. Adorno, R. Q. Barreto, M. dos Santos Ferreira, L. S. M. Lino, V. B. D. O Amorim, J. A. D. Santos-Serejo, F. Haddad, C. F. Ferreira, and E. P. Amorim. 2021. Pre-selection of banana somaclones resistant to *Fusarium oxysporum* f. sp. *cubense*, subtropical race 4. *Crop Protection*, 147, 105692.
- Segura-Mena, R. A., J. J. Stoorvogel, F. García-Bastidas, M. Salacinas-Niez, G. H. J. Kema, and J. A. Sandoval. 2021. Evaluating the potential of soil management to reduce the effect of *Fusarium oxysporum* f. sp. *cubense* in banana (*Musa AAA*). *European Journal of Plant Pathology*, 160(2), 441-455.
- Siamak, S. B., and S. Zheng. 2018. Banana Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*) control and resistance, in the context of developing wilt-resistant bananas within sustainable production systems. *Horticultural Plant Journal*, 4(5): 208-218.
- Staver, C., D. E. Pemsil, L. Scheerer, L. Perez Vicente, and M. Dita. 2020. Ex ante assessment of returns on research investments to address the impact of Fusarium wilt tropical race 4 on global banana production. *Frontiers in Plant Science*, 11, 844.
- Su, H. J., S. C. Hwang, and W. H. Ko. 1986. Fusarial wilt of Cavendish bananas in Taiwan. *Plant Disease*, 70(9), 814-818.
- Syed, R. N., A. M. Lodhi, M. M. Jiskani, K. I. Rajput, M. A. Khaskheli, M. A. Khanzada, N. A. Rajput, S. A. Maitlo, and A. Q. Rajput, 2015. First report of Panama wilt disease of banana caused by *Fusarium oxysporum* f. sp. *cubense* in Pakistan. *Journal of Plant Pathology*, 97(1).
- Thangavelu, R., M. Loganathan, R. Arthee, M. Prabakaran, and S. Uma. 2020. Fusarium wilt: a threat to banana cultivation and its management. *CABI Reviews*, (2020), 1-24.
- Thangavelu, R., M. Gopi, P. Pushpakanth, M. Loganathan, E. Edwin Raj, N. Marimuthu, M. Prabakaran and S. Uma. 2021. First Report of *Fusarium oxysporum* f. sp. *cubense* VCG 0125 and VCG 01220 of Race 1 Infecting Cavendish Bananas (*Musa* sp. AAA) in India. *Plant Disease*, 105(4), 1215-1215.
- Thangavelu, R., E. Edwinraj, M. Gopi, P. Pushpakanth, K. Sharmila, M. Prabakaran, M. Loganathan, and S. Uma. 2022. Development of PCR-Based Race-Specific Markers for Differentiation of Indian *Fusarium oxysporum* f. sp. *cubense*, the Causal Agent of Fusarium Wilt in Banana. *Journal of Fungi*, 8(1), 53.
- Viljoen, A. 2002. The status of Fusarium wilt (Panama disease) of banana in South Africa. *South African Journal of Science*, 98(7): 341-344.
- WU, K. L., W. Z. CHEN, S. YANG, Y. WEN, Y. R. ZHENG, W. M. Anjago, Y.Z. Yun and Z. H. WANG. 2019. Isolation and identification of *Fusarium oxysporum* f. sp. *cubense* in Fujian Province, China. *Journal of Integrative Agriculture*, 18(8), 1905-1913.
- Zhang, L., T. Yuan, Y. Wang, D. Zhang, T. Bai, S. Xu, Y. Wang, W. Tang, and S. J. Zheng, (2018). Identification and evaluation of resistance to *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in *Musa acuminata* Pahang. *Euphytica*, 214(7), 1-12.