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RESEARCH ARTICLE

Fusarium in banana from Ecuador: A morphological, molecular and pathogenic study as a control strategy

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Abstract

Fusarium oxysporum f. sp. *cubense* is associated with wilting in banana cultivation. The soil-dwelling phytopathogenic fungus has caused devastating consequences worldwide. The races that affect bananas are 1, 2 and 4, the latter very feared because of the epidemics caused since its identification, and because to date an effective control against the pathogen has not been determined. Therefore, the objective of the research was to isolate and characterize morphologically using four culture media, and molecularly the fungus in order to evaluate its pathogenicity with *in vitro* and greenhouse tests. 20 symptomatic samples were collected from an apple variety banana plantation. Subsequently, isolations were made from vascular tissue. The morphological identification of the isolates obtained was decisive to use a single strain for subsequent analysis and trials due to the similarity of the isolate's strains. These strains were characterized by a cottony white mycelium that gradually changed to purple. Microscopy identified macroconidia, microconidia and chlamydospores. Molecular analysis was performed through the genes: translation elongation factor TEF1α, and Rpb2. The strain (Fus banana) was identified as *F. oxysporum* f. sp. *cubense*, accessions TEF1α (PV682586), Rpb2 (PV682587). The results were expressed in terms of incidence and severity. The medium malt extract registered a higher incidence reaching 99%. Regarding the distribution of severity, a progressive pattern in the intensity of symptoms was observed. The results obtained constitute consistent scientific support for future studies that are oriented towards the development of reliable diagnostic tools, the evaluation of pathogenicity in banana materials and the design of integrated management strategies against this pathogen with high economic and phytosanitary impact.

Keywords: *Fusarium*; incidence; severity; TEF1α; Rgb2.

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1. Introduction

Bananas are one of the main crops worldwide. In 2023, the harvested area reached 5,973,253 hectares, with an estimated global production of 139,277,894.61 tons (FAOSTAT, 2023). In Ecuador, banana exports reached 337.31 million boxes in 2025, representing an increase of 3.45% compared to 2024 (AEBE, 2025). According to (FAO, 2020) projections, it is estimated that by 2028 Ecuador will consolidate its leadership, with an approximate volume of 7.4 million tons, which is equivalent to a third of the global market.

This crop represents an essential livelihood for smallholder farmers, as well as a significant source of export earnings. Banana production in the world is centered on the Cavendish variety (Dita et al., 2018), reporting more than 40% in global markets and almost all exports. This has intensified their vulnerability to pathogens such as *Fusarium oxysporum* f. sp. *cubense* and black Sigatoka (Drenth & Kema, 2021). Putting exports and subsistence production at risk (Strobl & Mohan, 2020).

Fusarium is characterized as a soil-dwelling pathogen (Agrios, 2005; Arie, 2019; Validov et al., 2011) that over time has caused great economic losses in the cultivation of musaceae. This genus has many strains, species, and metabolites that have affected agriculture (Ploetz, 2000). The pathogen produces microconidia, macroconidia, and chlamysdospores. It features a mycelium of white, pink to purple (García et al., 2011).

Fusarium oxyspurum f sp. *cubense* is a pathogen that is difficult to identify due to its genetic and physiological variability. Four races have been described: race 1 affects the varieties Gros Michel, Manzano among others, race 2 affects Bluggoe, Sapino, race 3 affects heliconias and race 4 is divided into subtropical SR4 and tropical R4T affects all Musaceae and also has special forms (Leslie & Summerell, 2006; Ploetz, 2000).

In 2019, the presence of Foc TR4 was confirmed in Colombia (Ayuso-Álvarez et al., 2020; Bakhat et al., 2023), in 2021 it was identified in Peru (Acuña et al., 2022; Bakhat et al., 2023), in 2023 the pathogen entered Venezuela (Bakhat et al., 2023; Mejías et al., 2022). Race 1 and race 4 are predominant in banana cultivation, affecting banana cultivation, causing significant economic losses (Nozawa et al., 2023). (Rodríguez-Yzquierdo et al., 2023) attribute that there are soil conditions that predispose to the disease, such as the physical-chemical properties of the soil.

Molecular identification is based on PCR and sequencing with molecular markers (TEF1 α, Rpb1, Rpb2) (Balajee et al., 2009; O'Donnell et al., 2015) which are specialized techniques, suggesting a problem. Specific primers have even been designed for confirmation. Developed differentiated *primers* for Foc 1 and Foc 4 (W1805F/W1805R and W2987F/W2987R, respectively), while (Pérez-Vicente et al., 2014) designed a *specific* primer (FocTR4-F/FocTR4-R), which allows discrimination between the TR4 and SR4 lineages. Magdama et al. (2019) mentions that there is a possibility of finding false positives in molecular diagnosis, due to the presence of non-pathogenic strains. Therefore, it is important to consider the biology, diversity and specificity of species in order to develop accurate and reliable diagnostic tools.

This study aimed to characterize *Fusarium oxysporum* morphologically and molecularly, as well as evaluate its pathogenicity using a standardized protocol that contributed to the early detection of the pathogen under field conditions. The information generated was essential for strengthening contingency plans and supporting the establishment of effective disease management strategies in banana production systems in Ecuador.

2. Methodology

2.1. Collection of samples of infected plant tissue

20 samples of plant tissue with symptoms of yellowing in leaves and necrosing of vascular tissue, typical of the wilting produced by *Fusarium* in the province of Los Ríos (0°51'42.8"S 79°22'49.7"W), were collected in a banana plantation, later, they were taken to the Agrobiotechnology laboratory of the Universidad San Francisco de Quito where the isolation of the phytopathogen was carried out.

2.2. Mushroom isolation

After the collection of 20 samples of infected plant tissue in the Los Ríos area (0°51'42.8"S 79°22'49.7"W), laboratory isolates were carried out, following the protocol described by Dhingra and Sinclair (1995). 2x2 mm cuts were made from the infected plant tissue, which were superficially disinfected and then sown in PDA (Potato Dextrose Agar- Difco) culture medium. Subsequently, 5 mm diameter discs containing the fungal spores were extracted and incubated for 7 days at 28 °C (Shell Lab). It was decided to use a single strain for all analyses due to the similarity of the isolates.

2.3. Morphological characterization

Once the pure strain was obtained, morphological characterization was carried out to determine the color (whitish that turns purple), shape of the colony (rounded), as well as the spores (macroconidia, microconidia and chlamydospores) (García-Bastidas et al., 2019). PDA (Potato Dextrose Agar), Malt Extract, SNA (Spezieller Nährstoffarmer Agar), Agar-Agar (Leslie & Summerell, 2006) were used. The fungal structures were observed by optical microscopy Leica DM750 using 40X and 100X lenses and compared with the descriptions reported in the literature. 60 spores, macroconidia, microconidia and chlamydospores were counted to assess size (length and width). At the same time, four culture media (SNA, PDA, Agar-Agar and malt extract) were evaluated to induce the production of different types of spores.

2.4. Pathogenicity testing

To climatize the seedlings received *in vitro*, they were transplanted in sterile substrate, kept for 12 weeks in a greenhouse at 26 °C, with weekly irrigation using distilled water and application of Hoagland nutrient solution. Subsequently, the Fusarium *inoculum was prepared* with a concentration of 1×10^7 mL^{-1} spores. Superficial wounds

were made on the roots, followed by the application of 5 mL of spore suspension per plant. Subsequently, the plants were immersed for 30 min in the remaining suspension. Eight days later, the application of a volume of 5 mL of sporulated solution was repeated (García-Bastidas et al., 2019). Pathogenicity was evaluated through Koch's Postulates (Agrios, 2005), and the presence of the pathogen was confirmed by isolation of symptomatic plants. The incidence and severity (using a scale of 1 to 4 for external leaf yellowing and internal rhizome discoloration symptoms) of the disease was measured weekly for eight weeks (García-Bastidas et al., 2019).

2.5. Molecular characterization

To carry out the molecular characterization, the fungus was cultured in liquid LB medium to facilitate DNA extraction. Qiagen's DNA Kit was used, according to the protocol determined by the manufacturer, PCR was performed with a final volume of 24 µL, to amplify the ITS (Internal Transcribed Spacer) region, the primers ITS 4 (5'-TCCTCCGCTTATTGATATG-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') were used. The TEF1a (Elongation factor 1 alpha) region was amplified using the primers EF-1 (5'-ATGGGTAAGGARGACAAGAC-3') and EF-2 (5'-GGARGTACCAGTSATCATGTT-3') primers (O'Donnell et al., 2015).

Amplification was performed using the following parameters: initial denaturation at 94 °C for 4 minutes, followed by 30 cycles of denaturation at 94 °C for 45 seconds, hybridization at 52 °C for 30 seconds, extension at 72 °C for 45 seconds, and a final extension at 72 °C for 15 minutes. On the other hand, the TEF1 amplified PCR program α was initial denaturation at 95 °C for 5 minutes, followed by 35 denaturation cycles at 94 °C for 1 minute, hybridization at 55 °C for 2 minutes, extension at 72 °C for 2 minutes, and a final extension at 72 °C for 10 minutes. The PCR products were separated by electrophoresis at 100V for 30 minutes in 2% agarose gel in 1x TBE buffer (O'Donnell et al., 2015). The amplified PCR products obtained were sent to MACROGEN in Korea for sequencing. The sequences obtained were compared with the BLAST (Local Search for Protein Alignments and Sequences) sequences in the NCBI (National Center for Biotechnology Information) database. The phylogenetic tree was made with the markers TEF1 α and Rpb2. MEGA was used to align and BEAST to run the tree, with Bayesian inference, using GTR+G+I, with an MCCM of 100000000.

2.6. Statistical analysis

Statistical analyses were performed using analysis of variance (ANOVA) under a randomized complete block design (RCBD) to evaluate the effect of treatments on disease incidence and severity. Disease incidence and severity were analyzed separately. Severity was assessed using an ordinal rating scale and subsequently converted into a severity index (%) prior to analysis. Data were checked for normality and homogeneity of variances, and percentage data were transformed when necessary. When significant differences were detected ($p \leq 0.05$), treatment means were compared using Tukey's Honestly Significant Difference (HSD) test. All analyses were conducted using SISVAR (Ferreira, 2019).

3. Results and discussion

3.1. Pathogen isolation and morphological characterization

In PDA medium, the strain of *Fusarium oxysprorum* f. sp. *cubense* reached 70 - 90 mm in diameter after 7 days at 28 ºC in darkness. The mycelium initially presented a cottony whitish mycelium, progressively turning purple, especially visible in the center of the colony as growth progressed (Figure 1). This morphology is consistent with what was described by Mejías et al. (2022) and Ploetz et al. (2015) who report colonies with dense aerial mycelium, white on the margins and pink or purple on the back and center, which is useful for a preliminary diagnosis.

It should be noted that temperature is a key factor for the development of the fungus and in morphological expression (Pérez-Vicente et al., 2014), established an optimal range of development ranging from 23 ºC - 27 ºC, which is aligned with the results obtained, even though growth was evaluated at 28 ºC, which proximity to the optimal range favored the vigorous development of mycelium and the typical expression of pigmentation. These findings reinforce the importance of environmental conditions to produce inoculum from subsequent assays.

3.2. Morphological characterization

At the microscopic level, characteristic reproductive structures of *Fusarium oxysporum* f. sp. were observed with abundant microconidia, scarce macroconidia and terminal chlamydospores. This morphological distribution is consistent with that described by Leslie & Summerell (2006) who point out that this pathogen usually presents microconidia abundant in monophylies, while macroconidia are rare, and chlamydospores occur in terminal and intercalary forms (Figure 2).

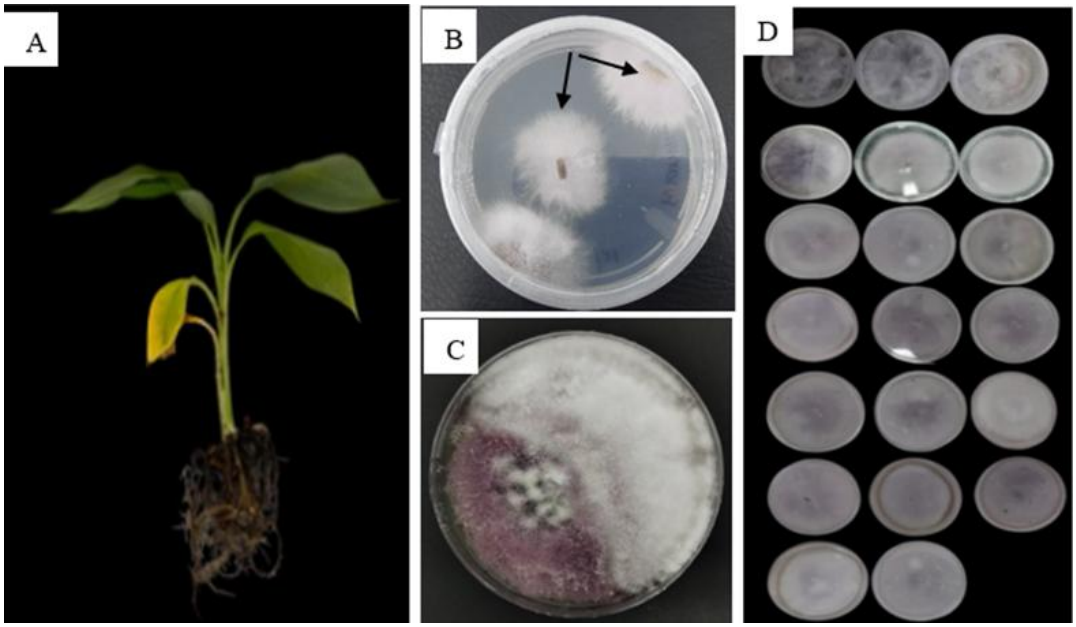

Figure 1. Isolates and morphology of *Fusarium* sp. A: Tissue with manifestation of vascular necrosis of dark brown coloration B: Growth of the fungus from infected material C: External characteristics of the isolation of *Fusarium* sp. D: *Fusarium* sp. Isolates.

The microconidia had an elliptical shape, and without septa. Its size (µm) ranged from 6.40-15.83 long by 2.98-3.51 wide. The maximum size recorded was 15.83*3.51 µm, and the minimum (6.40*2.98 µm). These results are consistent with what was reported by Baruah et al. (2025) who describe oval microconidia with 0-2 septa and dimensions ranging from (10-12*2.2-3.0 µm).

The macroconidia were observed with an elongated and curved shape, similar to a crescent. They presented between 3-4 septa, with sizes ranging from 27.24-40.02 µm long by 3.41-5.54 wide. The maximum size recorded was 40.02*5.54 µm, and the minimum was 27.24*3.41µm. These characteristics coincide with what was described by Baruah et al. (2025) who observed macroconidia of falcate shape and size between (27-30 *3.2-3.6 µm).

As for chlamydospores, they were characterized by being spherical with thicker walls. Its size varied between 7.2-11.2 µm, within the range reported by (Baruah et al., 2025), which indicates values between 7.5-9.5 µm. These findings support the classic morphological description of the *Fusarium oxysporum* species complex (FOSC) (García-Bastidas et al., 2019).

By evaluating the ability of the four-culture media to induce the production of sporulating structures typical of *Fusarium*. A high production of macroconidia and chlamydospores was observed in the ANS medium, making it an efficient medium for morphological identification. In contrast to the PDA medium, it favored moderate production of microconidia, lower structural diversity. On the other hand, the Agar-Agar medium induced a low production of microconidia. Finally, the malt extracts medium promoted abundant microconidia production (Figure 2). Leslie & Summerell (2006) point out how the culture medium significantly influences the production of sporulating structures of the fungus.

3.3. Pathogenicity testing

The pathogenicity test of *the Fusarium* isolates showed the appearance of symptoms from 28 days after inoculation. Initial symptoms included progressive yellowing of older leaves to younger ones, accompanied by lightning of veins. At 56 days after inoculation, symptoms intensified with the appearance of generalized leaf necrosis, and when cross-sectional sections were made, vascular discoloration of the corm was observed, suggesting an obstruction in the vascular bundles (Martínez et al., 2023; Purwati et al., 2008). Koch's Postulates were fulfilled by reisolating the pathogen from plants inoculated with wilting symptoms.

As for the incidence, 14.25% was recorded at 28 days and progressed towards 56 days with 94.25%. The severity of the disease was defined on a scale of 4 for internal and external symptoms, where 17.9% corresponded to Severity 1, 21.6% Severity 2, 28.4% Severity 3 and Severity 32.1% (Figure 3). Several studies of climate demonstrate the correlation between the incidence, severity of the disease and other factors such as soil, climate, management, however, a comprehensive understanding of these factors and their interactions is necessary (Olivares et al., 2021).

In the inoculation trials of *Fusarium oxysporum* carried out in two varieties Manzano and Cavendish, it was obtained that in Manzano there was an incidence of 83.2% and a severity of 74.4% while in the variety Cavendish the disease did not occur (Figure 4) these results suggest that the strain used for the trials is race 1, which affects varieties such as Manzano and Gros Michel, but Cavendish shows genetic resistance (Ploetz et al., 2015; Su et al., 2020). This finding contributes to suggesting that race 4 is not present in Ecuador.

3.4. Molecular characterization

The strain was coded as Fus banana, the identity of the species was verified through the analysis of the TEF1 α and Rpb2 sequences with the use of the BLAST database, it was determined that the isolate corresponds to *Fusarium oxysporum* , obtaining an identity percentage of 99.44% and 98.05% according to each primer, the sequence information was uploaded to the GenBank, obtaining the following accession codes (Table 1).

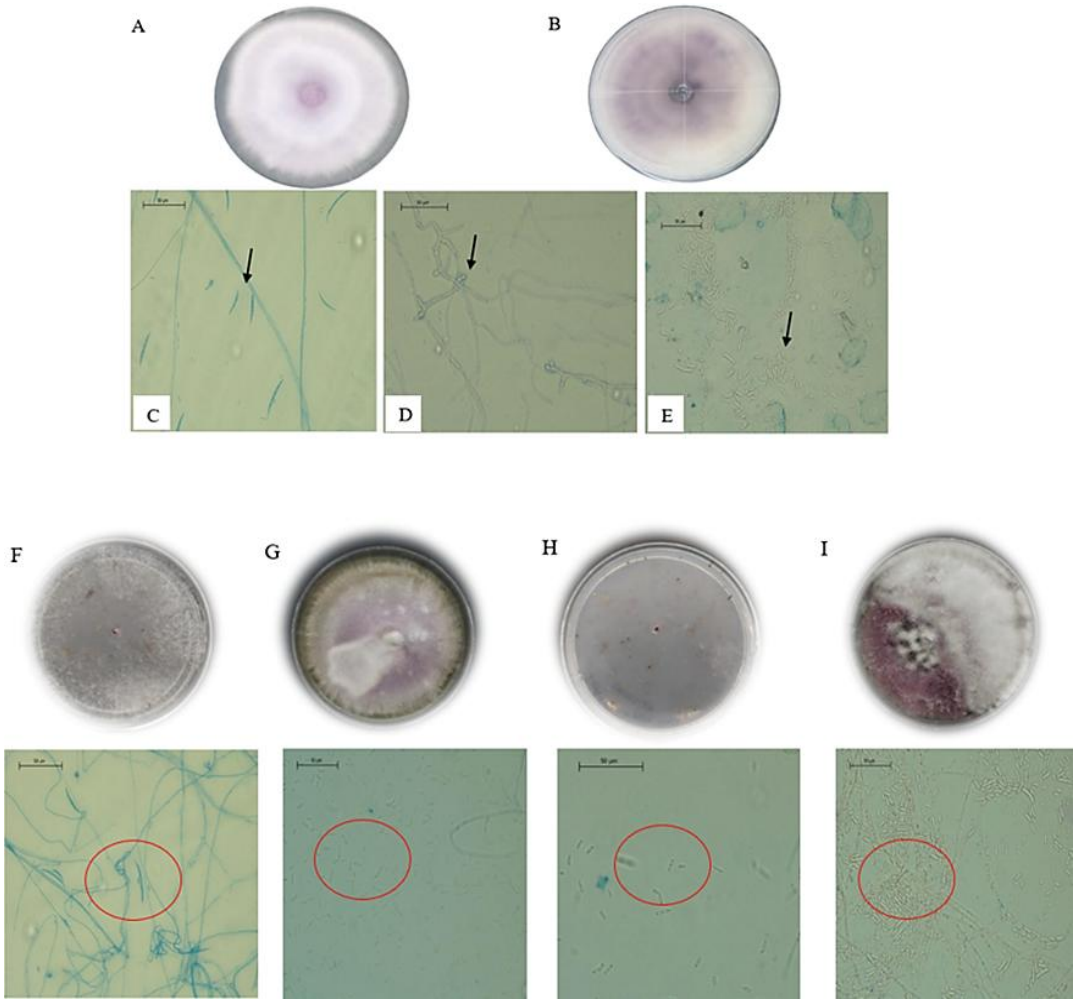

Figure 2. Fusarium microscopy A: Top view of white cottony mycelium B: Back view of purple cottony mycelium C: Macroconidia D: Chlamydospores E: Microconidia. Observation in 40X lens. Production of *Fusarium* spores in different culture media F: Culture medium SNA ((Spezieller Nährstoffarmer Agar), production of macroconidia and chlamydospores G: PDA medium (Potato Dextrose Agar), medium production of microconidia H: Medium Agar-Agar, low production of microconidia I: Medium malt extract production microconidia in large quantities.

Table 1
Molecular identification with ITS, TEF1 α and Rpb2 primers by means of BLAST

Sample	Scientific name	Region	Sequence Length	Query cover	Value	%Identity	GenkBank
Fus banana	*Fusarium oxysporum*	STIs	523bp	96%	0.0	99.44%	PQ897763
		TEF	276bp	99%	4E-120	98.05%	PV682586
		Rgb2	1065bp	100%	0.0	100%	PV682587

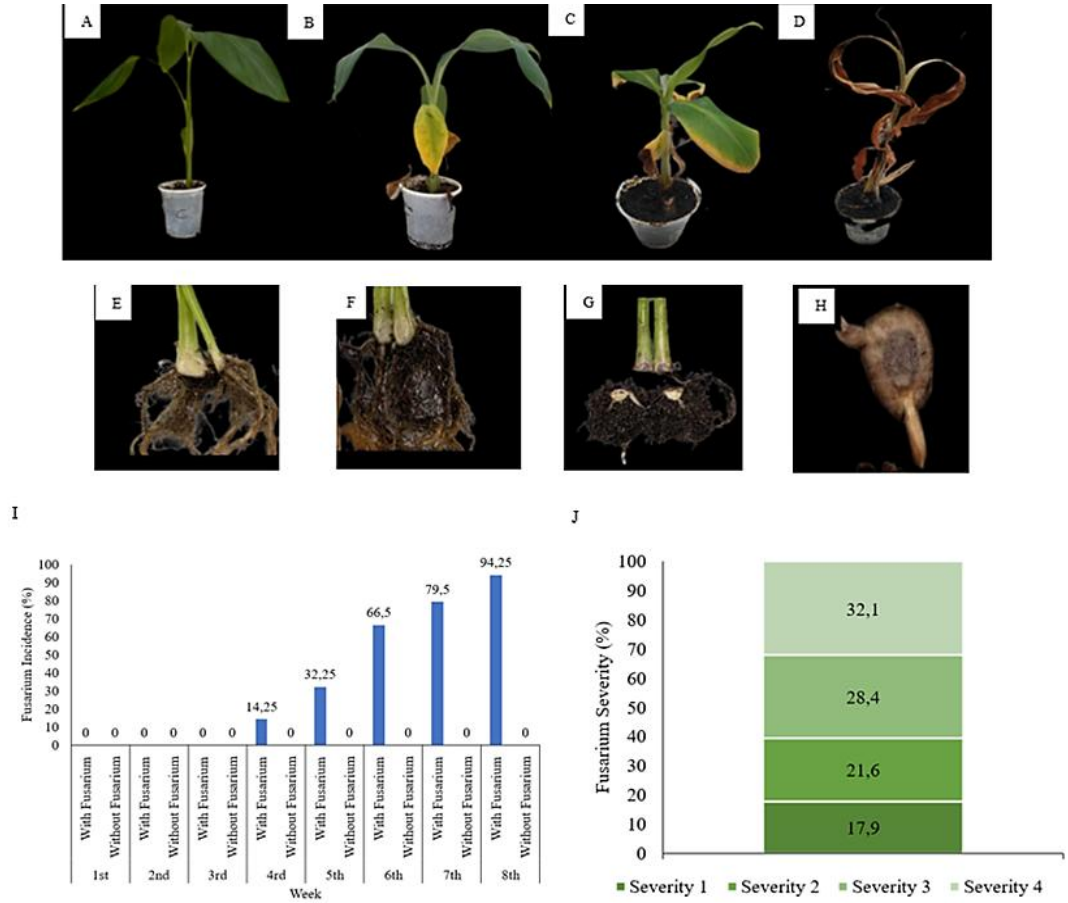

Figure 3. Pathogenicity of *Fusarium*. A, B, C, D: From left to right external symptoms of wilt, E, F, G, H: From left to right internal symptoms of wilt, I: Incidence (%) of *Fusarium*, J: Severity (%) of *Fusarium*, according to severity scale: Severity 1: Initial symptomatology, yellowing of the tip of the leaf (1 > x ≤ 25), Severity 2: Necrosis at the tip of the leaf and edges with yellowing (25 <x ≤ 50), Severity 3: Necrosis and yellowing of leaves (50 < x ≤ 75%), Severity 4: Necrosis and wilting (75 <x ≤ 100%).

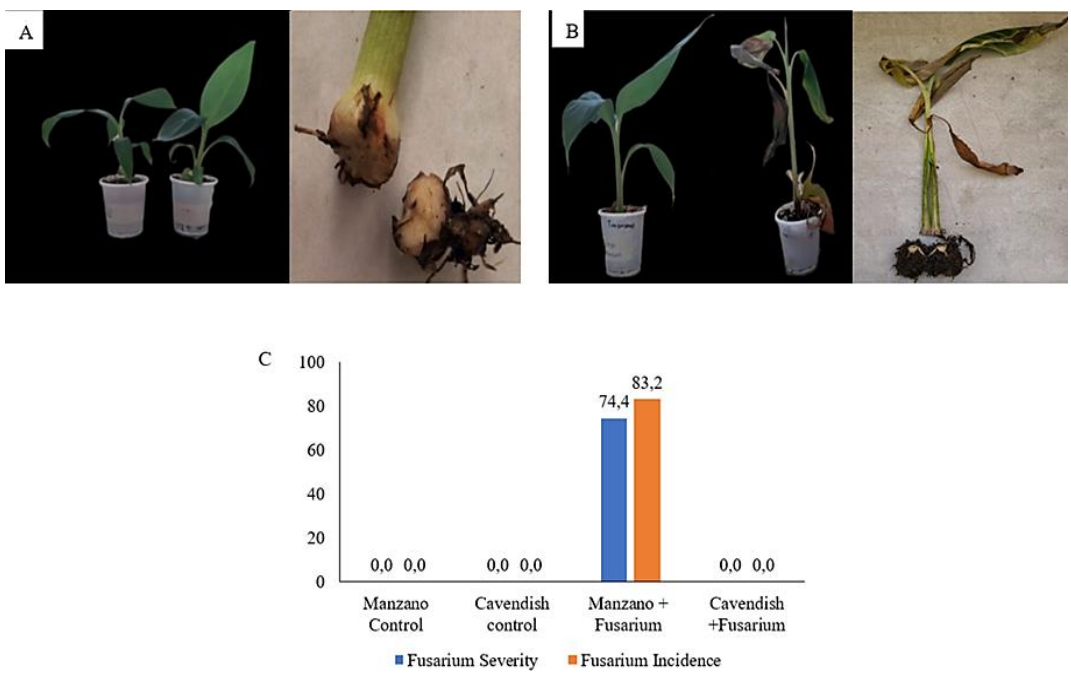

Figure 4. Fusarium pathogenicity. A: From left to right external and internal symptoms of wilt in the Cavendish variety, B: From left to right external and internal symptoms of wilt in the Apple variety, C: Severity and incidence of *Fusarium* in the Apple varieties.

The phylogenetic analysis, based on the TEF1 α and Rpb2 genes performed with Bayesian probability using the Markov Chain Monte Carlo (MCMC) algorithm for the Fus Banana sample (Figure 4), reveals a well-defined hierarchical structure grouping the strain into complexes (FOSC *Fusarium oxysporum* Species Complex; FFSC; *Fusarium fujikuroi* Species Complex; FSSC; *Fusarium solani* Species Complex, FIESC; *Fusarium incarnatum-equiseti* Species Complex) and FIBSC (*Fusarium* international biological species complex). These results are supported by high statistical probabilities in both Bayesian and maximum likelihood analyses, giving a clear hierarchical delimitation between complexes, placing the strain at the taxonomic level and reflecting deep evolutionary divergences (Achari et al., 2020).

The Fus banana strain is within the FOSC complex (*Fusarium oxysporum* species complex), it belongs to the complex of species that cause the wilt disease caused by *Fusarium* in bananas. A polyphyletic group that includes both pathogenic and non-pathogenic strains associated with a wide range of hosts (Ma et al., 2013). This complex is recognized due to its high genetic and phenotypic diversity; it comprises multiple special forms that show specificity towards certain plant species.

These complexes show clear genetic differences, reflected in the length of the branches and the values of statistical support. The bootstrap value and Bayesian posterior probability observed in the phylogenetic tree provide evolutionary support for the reliability of the grouping of the Fus Banana strain within the FOSC clade (0.99 bootstrap value) (Figure 5). This result is consolidated with described by Achari et al. (2020), since the Fus banana strain presents 99% Bootstrap values and posterior probability support (PP) greater than 0.90, this indicates a very high evolutionary confidence in its location within the clade. Finally, highlight that the combination of TEF1α and RPB2 in multilocus analysis significantly improves the accuracy in the identification and classification of *Fusarium species*, consolidating their phylogeny.

The *Fusarium tree* highlights specific subclades, such as clades 1 and 2 of the FOSC, which group strains related to *F. oxysporum* f. sp. cubense, a banana pathogen (Figure 5). The diversity observed within the subclades suggests possible phenotypic and genotypic differences, valuable characteristics for identifying new species that have been little studied. To confirm the identity of the pathogen, a PCR diagnosis was made. Molecular markers TEF1α (translation elongation factor 1-alpha) and Rpb2 (RNA polymerase II subunit 2) were used, which are highly informative for species-level identification, respectively (Singha et al., 2016).

4. Conclusions

Morphological and molecular characterization confirmed the identity of the Fus Banana strain as *Fusarium oxysporum* f. sp. *based* on its phenotypic profile in selective media, its pathogenic capacity in susceptible varieties such as Manzano and its phylogenetic grouping within the FOSC complex. This finding, supported by the multilocus analysis of the TEF1α and Rpb2 genes, is validated as an effective tool for intraspecific diversity of this complex, crucial for phytosanitary surveillance programs.

The results reveal that environmental conditions such as incubation temperature and type of culture medium significantly influence Fusarium morphology and sporulation, critical aspects for inoculum production and pathogenicity assessment. The high incidence and severity observed in the Manzano variety demonstrates the breed specificity of the pathogen, suggesting that *Fusarium* race 1 is predominant in Ecuador, with no evidence of the presence of Tropical race 4, confirming the need to maintain strict surveillance and biosecurity measures.

These findings provide a foundation for future research, expanding multilocus and genomic analyses to a broader collection of *Fusarium oxysporum* isolates from different banana-growing regions of Ecuador. This will allow for a better understanding of population structure, pathogenic variability, and potential evolutionary dynamics within the FOSC complex.

Furthermore, the standardized morphology and pathogenicity protocols validated in this study could be applied to resistance screening of local and introduced banana cultivars, as well as to the evaluation of biological and integrated management strategies. These efforts would strengthen early warning systems and contribute to the development of science- based phytosanitary policies aimed at preventing the introduction and spread of more aggressive races, including Tropical Race.

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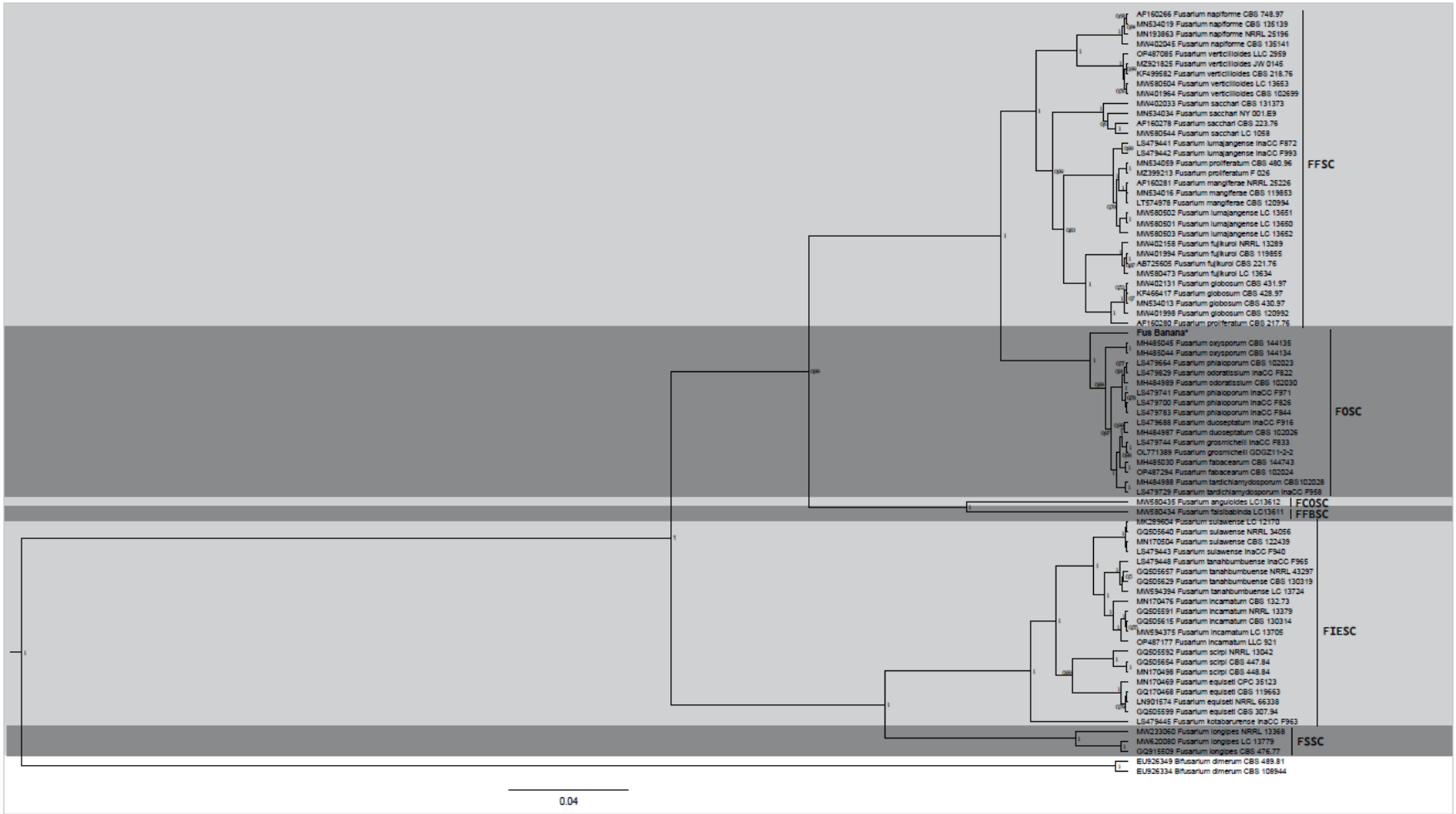

Figure 5. Concatenated phylogenetic analysis based on the TEF1 α and Rpb2 genes performed with Bayesian inference, using GTR+G+I, with an MCMC of 1000000000. External outgroup (*Beauveria dimerium*) was included to root the tree and Genbank sequences representative of major Fusarium species complexes.

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