

Research Article



Mapping and Molecular Detection of Banana Fusarium Wilt in South Sulawesi Using Volatile Odor Test and Race-Specific PCR Primers

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ABSTRACT

Fusarium wilt of bananas, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), poses a serious threat to banana cultivation, particularly with the emergence of physiological race 4. This study mapped the prevalence of Fusarium wilt in 16 governorates/cities of South Sulawesi Province (2020-2024) and identified physiological race 4 of Foc at various growth stages using odor detection (VOT) and PCR detection methods. Spatial and temporal analyses of regional disease prevalence were conducted at three sites (60-600 plants) in the Palangloe district of Gowa Regency. Samples were collected from symptomatic plants, inoculated onto PDA medium containing 500 ppm streptomycin, isolated on PDA, purified, and morphologically identified. The odor test was performed using a rice medium containing aldehyde odor indicators. DNA was extracted from meristem/apical and pseudostem/venous tissues and amplified using the primers FocEf3 (General Foc), SIX10a (Race 4), SIX13c (Race 4), and SIX1a (TR4) and visualized on a 2% agarose gel. Most areas exhibited very low infestation rates. Investigations revealed significant differences in infestation rates between different planting sites ($p < 0.001$), with the highest infestation rate (18.33%) in the Barangan cultivar of the Bellapunranga region and the lowest (0%) in the Raja/Kepok cultivar of the Borissalo Region. The aldehyde aroma produced by the isolates under VOT conditions was consistent with that of Foc Race 4. PCR results showed that the primer SIX1a_266 confirmed the presence of TR4 in two Cavendish samples from Bantokassi, highlighting the importance of interlayer monitoring and early stage seedling screening.



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1. Introduction

Fusarium wilt disease, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is one of the most destructive diseases threatening global banana production. It can infect banana plants at any stage of growth, from seedlings to mature plants, causing significant economic losses

to farmers. Since the 20th century, Fusarium wilt has caused significant damage to the global banana industry, destroying thousands of hectares of Gros Michel bananas in Central America. Although resistance to the Foc-1 physiological race in the Cavendish banana variety once saved the industry, the emergence of physiological race 4 (TR4) has once again threatened global banana production. TR4 threatens the banana industry and causes concern among hundreds of thousands of smallholder farmers in Asia and Africa (Drenth & Kema 2021). In

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Indonesia, the effects of physiological race 4 (TR4) have been observed in hundreds of hectares of Cavendish banana plantations in Lampung, threatening various local varieties, such as Ambon banana (*M. acuminata* var. Ambon), Bambulaja banana, Horn banana (*Musa* sp. var. Horn), Uli banana (*M. acuminata* var. Ketan), Kepok banana (*Musa* sp. var. Kepok), and Balang banana, all of which are highly susceptible to physiological race 4 (TR4) (Maryani *et al.* 2023).

To date, four races of *Fusarium oxysporum* f. sp. *cubense* (Foc) have been identified. Race 1, responsible for the 'Gros Michel' epidemic, also affects other varieties, such as 'Pome' AAB, 'Maqueno' Silk, and 'Pisang Awak' ABB. Race 2 targets mature ABB bananas, including 'Bluggoe' (AB). In contrast to races 1 and 2, which are limited to specific banana genomes, race 4 is the most virulent and infects all banana types susceptible to races 1 and 2 (Stover 1962; Bentley 1998). Foc race 4, also referred to as the tropical race, was initially reported in 1968 when it infected Cavendish bananas, which were previously considered resistant, in Taiwan. Subsequently, Foc race 4 was divided into subtropical race 4 (STR4) and tropical race 4 (TR4) groups. STR4 isolates cause disease in Cavendish in subtropical regions, whereas TR4 isolates are pathogenic to Cavendish under both tropical and subtropical conditions (Dita *et al.* 2018). Subtropical race 4 (STR4) comprises VCG 0120, 01209, 01211, and 01215, which affect Cavendish in subtropical areas. In contrast, tropical race 4 (TR4), such as VCG-01213/16, infects some of the same varieties as STR4, but only in the absence of the disease-inducing conditions found in subtropical regions. Race 3 is a pathogen of *Heliconia* spp. and is no longer regarded as a banana pathogen (Ismaila *et al.* 2023). Furthermore, several commercially cultivated bananas are known to be susceptible to Fusarium wilt disease caused by Foc TR4, including Pisang Ambon Kuning (Rahmi-Anna *et al.* 2014), Pisang Raja (AAB), Pisang Barangan (AAA), Pisang Kepok (ABB), and Pisang Siem (ABB) (Jumjunidang *et al.* 2012). The wild banana *Musa acuminata* var. *malaccensis* is resistant to Fusarium wilt disease (Kayat *et al.* 2009), whereas the wild banana *M. acuminata* var. *zebrina* and *M. acuminata* var. *banksii*, as demonstrated by tests conducted by Garcias-Bastidas & Kema (2014).

Foc is a highly complex pathogen, and several methods have been used to study its characteristics, including volatile compound production and PCR analysis. Characterization of *Fusarium oxysporum* f. sp. *cubense* Race 4 can be identified using the volatile

odor test (VOT) method. Pure cultures of Foc on rice medium produce an aldehyde aroma that characterizes the tested isolate as Foc race 4 (Nasir *et al.* 2003). However, the race identity of field-origin *F. oxysporum* isolates and their ability to infect banana plants remain unknown. Identification of *F. oxysporum* and its various races can be performed using specific primers FocEf3_F/FocEf3_R for all Foc (Widinugraheni 2018), SIX10a_309_F/SIX10a_309_R (Race 4) (Carvalhais *et al.* 2019), SIX13c_343_F/SIX13c_343_F (Race 4) (Carvalhais *et al.* 2019), SIX1a_266_F/SIX1a_R (TR4) (Carvalhais *et al.* 2019).

This study aimed to analyze the distribution of Foc in 16 districts in South Sulawesi and identify Foc races using the volatile odor test (VOT). Additionally, this study aimed to detect the early presence of Foc using PCR with four specific primer sets: (FocEf3_F/FocEf3_R) to detect Foc in general, (SIX10a_309_F/SIX10a_309_R) for detecting Foc race 4, (SIX13c_343_F/SIX13c_343_F) for race 4, and (SIX1a_266_F/SIX1a_266_R) for detecting TR4.

2. Materials and Methods

2.1. Materials Surveillance and Sample Collection

The data in this study were divided into two parts: data on the incidence of Fusarium wilt disease in South Sulawesi over five years (2020-2024) were obtained from two main sources: the Indonesian Central Statistics Agency and the Food Crops and Horticulture Protection Agency. The BPS provides total crop data for several districts, whereas the Food Crop and Horticulture Protection Agency provides records of Fusarium wilt disease cases (number of infected plants) in South Sulawesi. Data were collected from South Sulawesi, focusing on districts with documented outbreaks of Fusarium wilt. These data were processed to estimates of the incidence in each region.

The second data source was based on the results of a field survey. A field survey was conducted on banana plantations located in Belapunranga and Bontokassi villages, Lanna Subdistrict, Parangloe District, Gowa Regency. At each location, the population of plants observed varied from 60 to 600 banana plants, depending on the size of the observation area. The incidence of Foc was calculated as the percentage of plants showing Foc symptoms out of the total number of plants observed, using the disease incidence formula according to (Sitepu *et al.* 2014), as follows:

$$\text{Disease incidence \%} = \frac{\text{Number of symptomatic plants}}{\text{Number of plants observed}} \times 100\%$$

Sampling was conducted using purposive sampling method, which entailed the intentional selection of symptomatic plants by observing disease manifestation in the field. This process involves identifying symptoms of Fusarium wilt in plants, such as yellowing of the lower leaves and splitting of the pseudostem. In severe cases, the lower leaves may partially detach, and the stems, when cut crosswise and lengthwise, exhibit discoloration.

Subsequently, the Foc pathogen was isolated in the laboratory. Vascular strands from desiccated pseudostems were sectioned into 0.5-1 cm fragments and inoculated onto potato dextrose agar (PDA) supplemented with 500 ppm streptomycin as an antibacterial agent. This culture was incubated at room temperature for 2-3 days, and colonies exhibiting a pink hue were selected as wild strains of *Fusarium oxysporum* f.sp. *cubense*.

2.2. Sample Collection for PCR

Banana plant samples were systematically collected from three distinct growth stages: (1) plantlets derived from tissue culture laboratories, (2) acclimated plants in nurseries, and (3) mature plants in the field. For early detection, meristem and apical tissues were sampled from plantlets and plants in the acclimatization phase, whereas mature plants were sampled from pseudostem and midrib tissues for further analysis. This sampling

methodology was based on the anticipated accumulation of Foc, which is known to occur in various tissues throughout plant growth stages.

2.3. Volatile Odor Test

A volatile odor test was used to identify specific Foc race 4. This method is more efficient and has been tested previously. The results of the race study based on VOT were identical to those of VCG testing (Moore *et al.* 1995) and DNA fingerprinting (Bentley *et al.* 1998). This procedure involved implanting 0.5 cm of Foc colonies from purified isolates into rice medium (Nasir *et al.* 2003). Subsequently, the isolates designated for identification were inoculated into Erlenmeyer flasks containing a rice-based medium and incubated. The resulting cultures emitted fermented aldehyde aromas, which were categorized as odoratum and inodoratum.

2.4. DNA Extraction and Polymerase Chain Reaction

Total genomic DNA was extracted using a Plant Genomic DNA Mini Kit (Geneaid, Taiwan), following the manufacturer's protocol with some modifications. Steel beads (2 mm) were added to the lysis buffer to facilitate the mechanical disruption of banana tissue using a tissue homogenizer.

The extracted DNA samples were tested for the presence of Foc using PCR with specific primers for Foc and Foc Race 4 (Table 1) as follows:

Table 1. Markers and primer sequences used in this study

Primer	Primer sequence (5' to 3')	Target race	Length (bp)	Reaction conditions	References
FocEf3_F	CGTTCTGGCAC	Foc	600	94°C, 5 min; 94°C, 30 s, 57°C, 30 s, 72°C, 2 min 30 s, 30 cycles; 72°C, 5 min	(Widinugraheni <i>et al.</i> 2018)
FocEf3_R	GATTAATTCAC GGGGGGGAGAA TTAACGAAC				
SIX10a_309_F	CCACTGGCACC	Race 4	309	95°C, 3 min; 95°C, 15 s, 58°C, 15 s, 72°C, 1 min, 30 cycles; 72°C, 10 min	(Carvalhais <i>et al.</i> 2019)
SIX10a_309_R	AAAGACTTG CGATGCGGAGT ACTGGTTGA				
SIX13c_343_F	CAGCCTCCTAG	Race 4	343	95°C, 3 min; 95°C, 15 s, 58°C, 15 s, 72°C, 1 min, 30 cycles; 72°C, 10 min	(Carvalhais <i>et al.</i> 2019)
SIX13c_343_R	CGTCGAAAA CCGTGATGGGG TACGTTGTA				
SIX1a_266_F	GTGACCAGAAC	TR4	266	95°C, 3 min; 95°C, 15 s, 55°C, 15 s, 72°C, 1 min, 30 cycles; 72°C, 10 min	(Carvalhais <i>et al.</i> 2019)
SIX1a_266_2R	TTGCCACA CTTTGATAAGCAC CATCA				

2.5. Visualization and Analysis of Polymerase Chain Reaction

PCR products were separated by electrophoresis on a 2% agarose gel stained with FloroSafe DNA stain (1st BASE, Singapore) and visualized under ultraviolet (UV) transillumination (Gelato, China). The presence of a DNA band of the target size was considered as positive Foc detection.

3. Results

3.1. Incidence of Fusarium Wilt Disease

3.1.1. South Sulawesi Regional Data

The data on the incidence of Fusarium wilt disease per district in South Sulawesi for the period 2020-2024, as follows:

Based on data on the classification of Fusarium wilt disease incidence in districts/cities in South Sulawesi Province for the period 2020-2024 (Table 2), the incidence rates varied between regions. There are three regional classifications (red, yellow, and green) (Figure 1), with areas included in the green zone (very low incidence group, 0.000-0.100) dominating most of the province, particularly in the central and western parts, such as Luwu Timur, Luwu, Luwu Utara, Bulukumba, Pangkep, Sidrap, Pindrang, Sinjai, Soppeng, Gowa, Barru, and Bone. These green zones indicate that, in general, the incidence of Fusarium wilt is still relatively controlled in most banana cultivation centers in South Sulawesi. Conversely, areas with yellow zones (moderate incidence

group, 0.101-1.000) were concentrated in the northern and eastern parts, especially in Kota Palopo and Maros. The existence of yellow zones indicates areas with increasing incidence rates that require special attention, both in terms of cultivation management, land sanitation, and routine disease monitoring, to prevent it from developing into a more widespread outbreak. Meanwhile, the red zone (high incidence group, 1,001-40,000) appears to be relatively limited spatially but significant epidemiologically, especially in areas such as Pare-pare, and Selayar. These red zones represent areas with the highest incidence rates and have the potential to become sources of pathogen spread to surrounding areas if strict control measures are not implemented.

3.1.2. Field Survey at Observation Sites

Field surveys conducted at the three locations revealed a relatively low incidence of Foc, as shown in Table 3.

According to the findings (Table 3), the Borisallo location (Raja and Kepok) exhibited the lowest incidence, with zero cases (0.00%). This was followed by the Bontokassi location (Cavendish) and the Belapunranga location (Raja), each reporting only one case, corresponding to proportions of 0.17% and 1.67%, respectively. In contrast, a notably higher incidence was recorded at the Belapunranga observation site, particularly in Barangan cultivation, which accounted for 11 cases (18.33%), representing the highest proportion among all groups. Kepok cultivation at the same location reported two cases (3.33%). The chi-square test yielded a p-value

Table 2. Incidence Fusarium wilt in South Sulawesi

District	Incidence (%)				
	2020	2021	2022	2023	2024
Barru	0.000	0.000	0.001	0.000	0.000
Bone	0.000	0.009	0.004	0.000	0.000
Bulukumba	0.000	0.000	0.231	0.005	0.070
Gowa	0.004	0.003	0.003	0.000	0.007
Kota Palopo	0.000	0.602	0.000	0.000	1.928
Luwu	0.209	0.082	0.007	0.021	0.028
Luwu Timur	0.000	0.002	0.045	0.114	0.208
Luwu Utara	0.079	0.215	0.026	0.013	0.007
Maros	0.415	0.564	0.209	0.062	0.107
Pangkep	0.000	0.000	0.134	0.048	0.000
Pare-Pare	1.084	1.812	33.483	0.000	0.000
Pinrang	0.010	0.014	0.019	0.015	0.000
Selayar	0.000	0.000	5.736	18.982	3.930
Sidrap	0.000	0.021	0.039	0.014	0.000
Sinjai	0.000	0.000	0.056	0.000	0.000
Soppeng	0.023	0.000	0.000	0.000	0.000
Total	1.82	3.32	39.99	19.27	6.29

Processed data on disease incidence in 2026

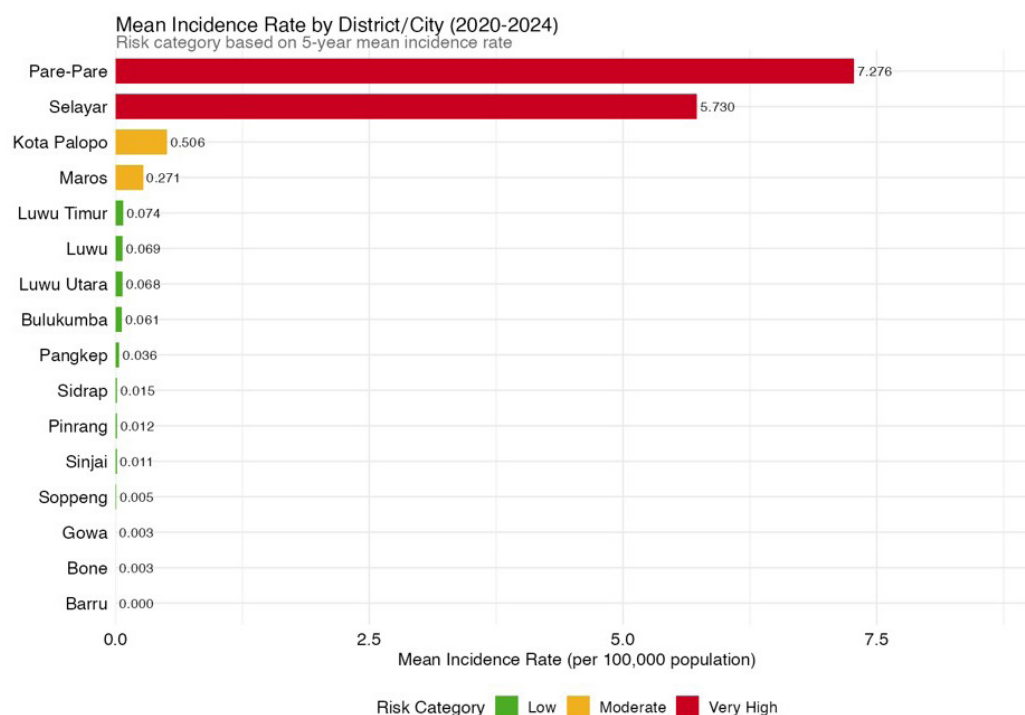


Figure 1. Disease incident zones based on the average incidence in each district (2020-2024)

Table 3. Incidence of Fusarium wilt disease

Place	Cultivar	Population	Infected plants	Disease incidence (%)	p-value
Bontokassi	Cavendish	600	1	0.17	<0.001
Belapunranga	Kepok	60	2	3.33	
Belapunranga	Barangan	60	11	18.33	
Belapunranga	Raja	60	1	1.67	
Borisallo	Raja dan Kepok	100	0	0	

SPSS *Chi-Square test*, 2026

of 0.001, indicating a statistically significant difference in the proportion of infection across the cultivation location groups. Consequently, the incidence of Fusarium wilt was unevenly distributed, with the Belapunranga Barangan group identified as having a relatively higher risk, thereby warranting prioritization for further control and monitoring.

Characteristic symptoms of Fusarium wilt were observed in the field, including yellowing of the lower leaves and splitting of the pseudostems. In severe cases, the lower leaves of the plant partially broke, and the stems that were split crosswise and longitudinally revealed the inside of the pseudostem, showing reddish, brownish, blackish, or yellowish vascular lines or threads, as shown in Figure 2.

Fusarium wilt initially manifests on older leaves as yellowish-green spots, beginning at the leaf margins

and progressing to younger tissues of the plant. Newly emerged leaves occasionally remain asymptomatic. Subsequently, the pseudostem exhibits fissures that are several centimeters above the soil surface. Fusarium wilt is also observed in young plants or seedlings, where suckers become diminutive, leaves appear narrower, and pseudostems develop cracks and expand upward. Upon sectioning the interior of the pseudostem and leaf stalks, tissue discoloration may be evident, potentially presenting as brown, black, purple, or yellow lines.

3.1.3. Isolation and Morphological Characterization

Morphological characterization revealed several unique traits that varied in terms of growth rate, colony color, and number of macroconidia (Figure 3). Macroconidia are four to eight-celled, sickle-shaped, thin-walled, and

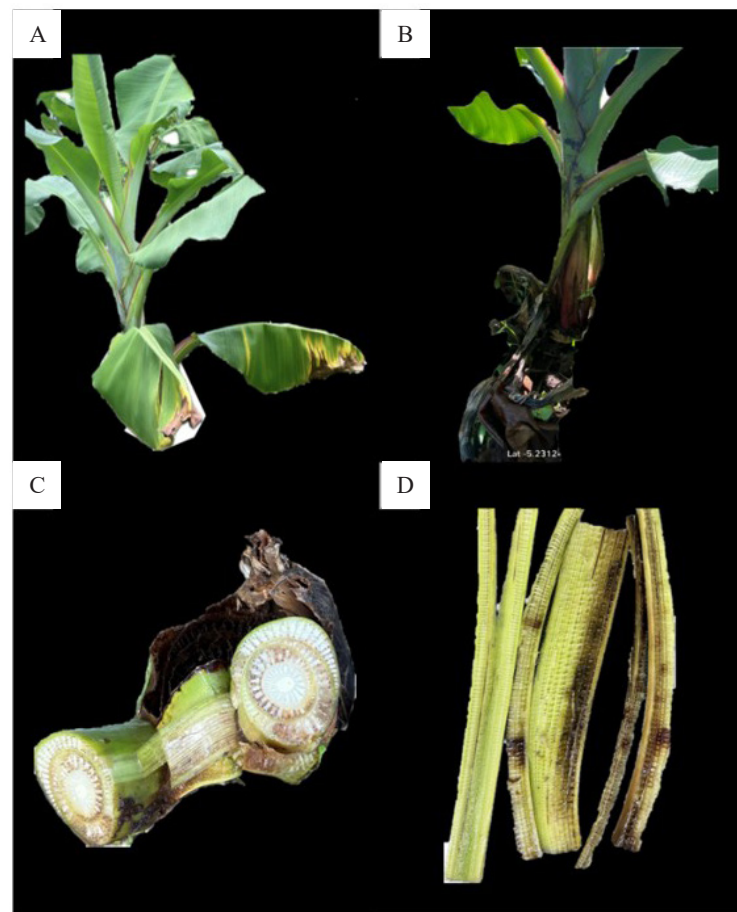


Figure 2. Symptoms of *Fusarium* wilt disease on various parts of banana plants: (A) yellowing of lower leaves, (B) breakage of the pseudostem, (C) symptoms on infected banana plant stems, (D) vascular symptoms in pseudostem tissue

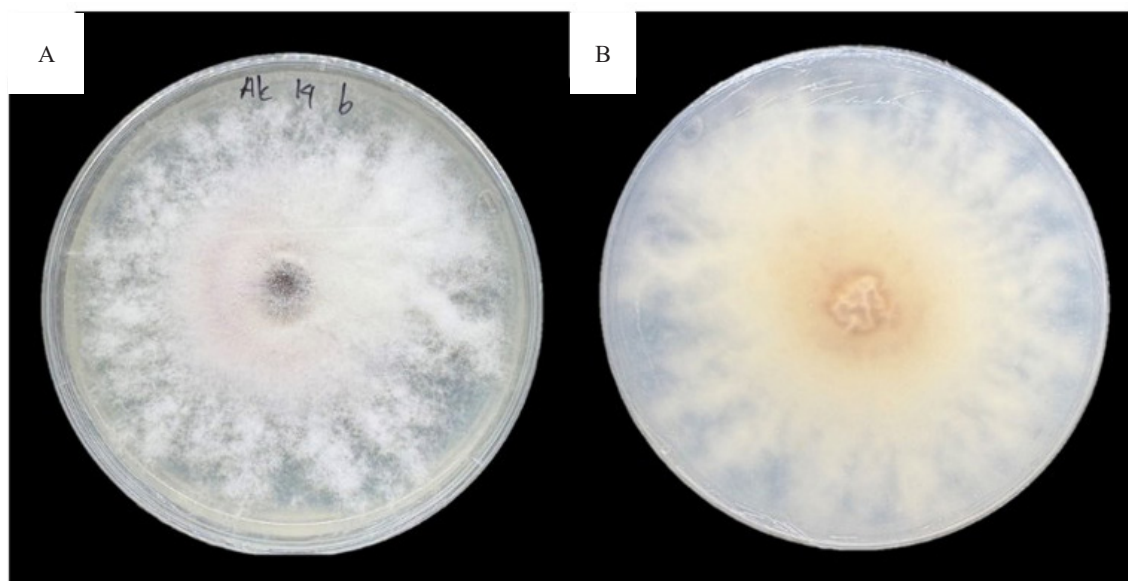


Figure 3. (A) Obverse view of *Fusarium oxysporum* f. sp. *cubense* isolate on PDA medium, (B) Reverse view of *Fusarium oxysporum* f. sp. *cubense* isolate on PDA culture medium

delicate. These spores are commonly found on the surfaces of plants killed by the fungi. Microscopic observation revealed the distinctive characteristics of the fungal species *F. oxysporum* f.sp. *cubense* (Figure 4), which can be observed from the shape and size of the macroconidia and microconidia. Chlamydospores are spherical, either individually or in groups, and are formed at the ends or in the middle of hyphal strands.

3.1.4. Volatile Odour Test

In pure Foc cultures cultivated on rice medium (Figure 5), two distinct colorations were observed: isolates from Cavendish exhibited a reddish hue, whereas those from Kapok and Raja exhibited a purple coloration. However, as noted by Nasir *et al.* (2003), color does not influence the test outcomes unless an aldehyde aroma is present. During the purification of isolates, six samples from a commercial tissue culture facility's plantlet specimens, as well as pseudo-stems from the Belapunranga, Kepok Selayar, and Susu Sudiang cultivars, could not be purified because of significant bacterial contamination. Following a three-day incubation period, fermentation results revealed the presence of aldehyde compounds in 10 of the 16 pure isolates from Cavendish, Kepok, and Raja, as indicated by a fermentation aroma, as shown in Table 4. This fermentation process demonstrates the activity of microorganisms in converting substrates to fermentation products. The formation of aldehydes during fermentation signifies enzymatic activity that breaks down complex compounds into simpler ones.

3.2. Polymerase Chain Reaction (PCR)

Pcr detection result across all growth phases are summarized in Table 5 and Figure 6. Detection ability differed according to the specificity of each primer target in the plantlet, acclimatization, and mature plant phases originating from tissue cultures. Primers (SIX10a_309_F and SIX10a_309_R) and (SIX13c_343_F and SIX13c_343_F) with the race 4 target showed no detection results (0%) in the early stages and in tissue culture plants. The primers FocEf3_F and FocEf3_R), which target Foc in general, detected only 5.3% of mature plant samples from tubers/shoots, indicating that detection with these primers requires a higher concentration of target DNA. Primers (SIX1a_266_F and SIX1a_266_R) targeting a specific Foc TR4 confirmed the presence of TR4 in 5.3% of samples (two Cavendish Bontokassi samples).

3.2.1. Electrophoresis Visualization Results

In the agarose gel of field samples (A), there were two samples that showed DNA bands at the 600 bp position, while Plantlet and Aklim samples (B), regional samples (C) and other field samples (A) did not show any DNA bands using the FocEf3_F/FocEf3_R primers for detection (Figure 7).

Positive detection using the SIX1a_F/SIX1a_R primers revealed DNA bands exclusively in two Cavendish samples from Bontokassi, demonstrating a sensitivity of 5.56% and confirming the specific presence of TR4 at the site, as show in Figure 8. This finding aligns with the results obtained using the FocEf3_F/FocEf3_R primers in the same samples, suggesting that co-detection using different markers enhances the accuracy of TR4 diagnosis.

4. Discussion

Based on the results of mapping the incidence of Fusarium wilt disease in 16 districts/cities of South Sulawesi during the period 2020-2024, it shows significant spatial heterogeneity, with most areas still in the green zone category (very low incidence, 0.000-0.100%), which includes banana production center areas such as Luwu, North Luwu, East Luwu, Bulukumba, Gowa, Bone, Sinjai, Soppeng, Pangkep, Sidrap, Pinrang, and Barru. However, two areas are included in the red zone (high incidence), Pare-Pare and Selayar. Pare-Pare recorded a striking spike in incidence in 2022, reaching 33.48%, and then dropped drastically to 0% in 2023 and 2024. Meanwhile, Selayar Regency showed a consistent upward trend, from 5.74% in 2022 to 18.98% in 2023, although it decreased to 3.93% in 2024. The high incidence in Selayar needs serious attention because this area has the potential to be a source of inoculum that can spread to other areas. Palopo City, which is included in the yellow zone, also needs to be monitored, especially with a surge in incidence in 2024 which reached 1.93% after previously not being detected in 2022 and 2023.

These sharp fluctuation patterns are most likely influenced by environmental conditions that support pathogen development in a given year, such as high humidity, rainfall, or improper cultivation practices. This is supported by Olivares *et al.* (2021), who stated that agroecosystem management plays a major role in the occurrence of disease events, especially Fusarium wilting. For example, plantations with a higher plant

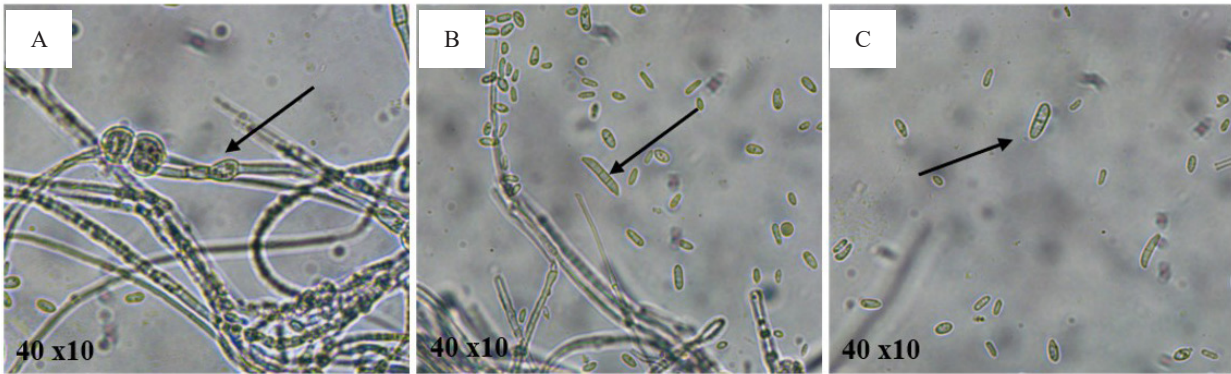


Figure 4. (A) Chlamydospores in the *Fusarium oxysporum* f. sp. *cubense* isolate, (B) Macroconidia in the *Fusarium oxysporum* f. sp. *cubense* isolate, (C) Microconidia in the *Fusarium oxysporum* f. sp. *cubense* isolate at 40×10 magnification

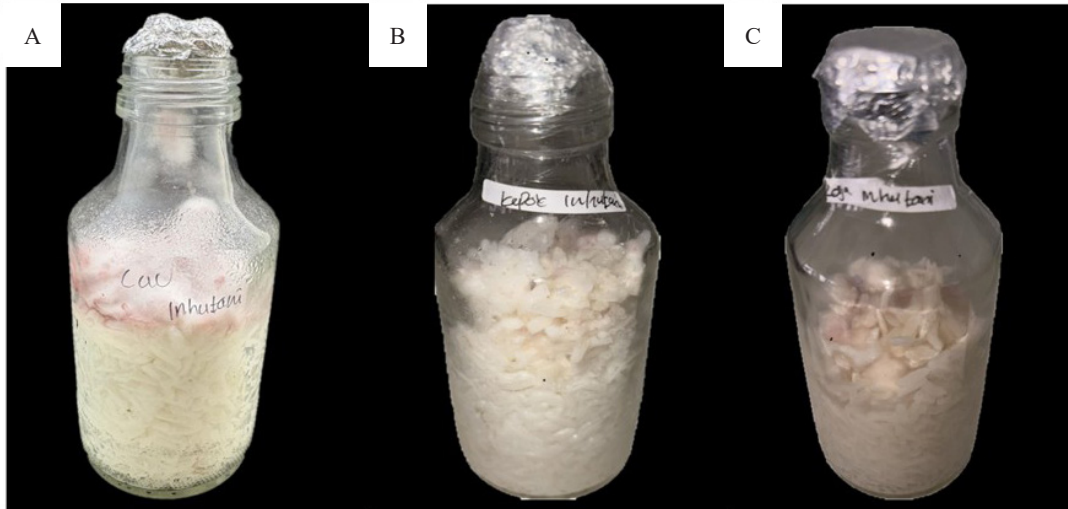


Figure 5. (A) Pure culture isolates from Cavendish plant samples, (B) Pure culture isolates from Kepok plant samples, (C) Pure culture isolates from Raja plant samples

Table 4. Volatile odor test result

Cultivar	Growth phase	Adehhyde aroma (Odoratum/Inodoratum)	Race	Region
Cavendish	Mature plant	Odoratum	4	Bontokasi
Cavendish	Mature plant	Odoratum	4	Bontokasi
Kepok	Mature plant	Odoratum	4	Belapunranga
Kepok	Mature plant	Odoratum	4	Belapunranga
Kepok	Mature plant	Odoratum	4	Belapunranga
Raja	Mature plant	Odoratum	4	Belapunranga
Barangan	Mature plant	Contaminated	-	Belapunranga
Barangan	Mature plant	Contaminated	-	Belapunranga
Cavendish	Plantlet	Contaminated	-	Biotechnology laboratory
Cavendish	Plantlet	Contaminated	-	Biotechnology laboratory
Cavendish	Acclimatized plant	Odoratum	4	Nursery
Cavendish	Acclimatized plant	Odoratum	4	Nursery
Cavendish	Acclimatized plant	Odoratum	4	Nursey
Kepok	Mature plant	Contaminated	-	Selayar
Susu	Mature plant	Contaminated	-	Makassar
Kepok	Mature plant	Odoratum	4	Makassar

The isolate did not grow and was heavily contaminated with bacteria

Table 5. PCR detection results

Growth phase	Cultivar	Location	Total sample	FocEf3		SIX_10 a		SIX_13 c		SIX1a	
				+	-	+	-	+	-	+	-
Planlet	Cavendish	Biotechnology laboratory	11	0	11	0	11	0	11	0	11
	Barangan	Biotechnology laboratory	5	0	5	0	5	0	5	0	5
Acclimatization	Cavendish	Nursery	16	0	16	0	16	0	16	0	16
Mature Plants from Tissue Culture	Cavendish	Moncongloe	5	0	5	0	5	0	5	0	5
	Cavendish	Bontokasi	3	2	1	0	3	0	3	2	1
	Cavendish		1	0	1	0	1	0	1	0	1
	Raja	Belapunranga	4	0	4	0	4	0	4	0	4
	Barangan		12	0	12	0	12	0	12	0	12
Mature plants originating from tubers/shoots	Kepok		4	0	4	0	4	0	4	0	4
	Ambon		1	0	1	0	1	0	1	0	1
	Raja	Sudiang	1	0	1	0	1	0	1	0	1
	Kepok		1	0	1	0	1	0	1	0	1
	Susu		1	0	1	0	1	0	1	0	1
	Taiwan	Pattalasang	1	0	1	0	1	0	1	0	1
	Raja		1	0	1	0	1	0	1	0	1
	Kepok	Pattalasang	1	0	1	0	1	0	1	0	1
	Tinggi Giant		1	0	1	0	1	0	1	0	1
	Kipas	Bone	1	0	1	0	1	0	1	0	1
	Siger Lampung		1	0	1	0	1	0	1	0	1
	Cavendish	Jeneponto	1	0	1	0	1	0	1	0	1
	Kepok	Selayar	1	0	1	0	1	0	1	0	1

PCR detection results 2026

Fusarium Detection by Growth Stage Using Four Primer Sets

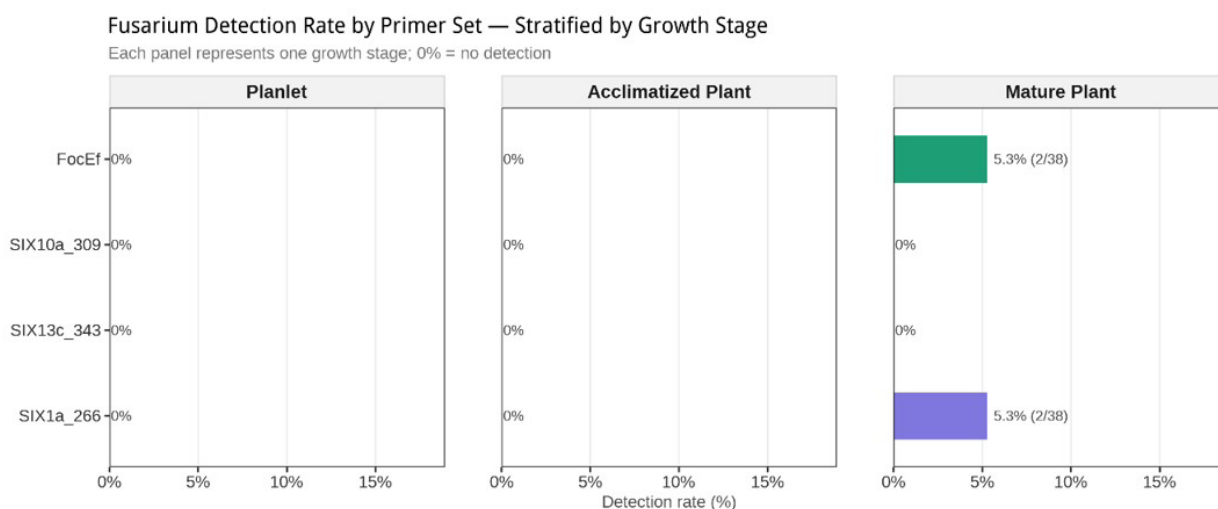


Figure 6. Primers (FocEf3_F/FocEf3_R), (SIX10a_309_F/SIX10a_309_R), (SIX13c_343_F/SIX13c_343_R), and (SIX1a_266_F/SIX1a_266_R) were used to detect various growth phase samples

density (< 3 m planting distance), cause an increase in plant infections. Because the smaller distance between banana plants has a negative impact on their health through competition. In addition, (Olivares *et al.* 2021) added that the physical and chemical properties characteristics of the soil and plant management have a major role in the

incidence of fusarium wilt disease, several soil chemical properties including pH, organic matter content and the availability of micronutrients in the soil are related to the repressive capacity against Foc pathogens. According to Rodríguez-Yzquierdo *et al.* (2023) states that areas classified as highly vulnerable to Foc TR4 have soil with

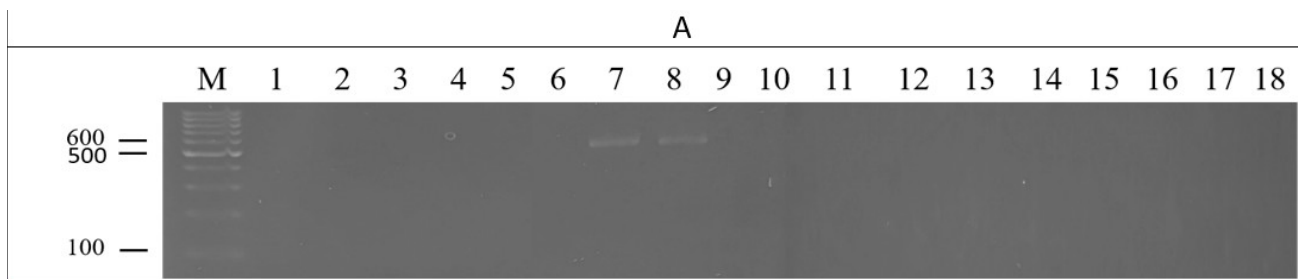


Figure 7. Electrophoresis results of DNA samples using primers FocEf3_F and FocEf3_R with a target amplification product of 600 bp: (A) Field samples. (1, 2, 3: Raja samples), (4, 5, 6: Kepok samples), (7, 8, 9: Cavendish samples), (10-18: Barangan samples)

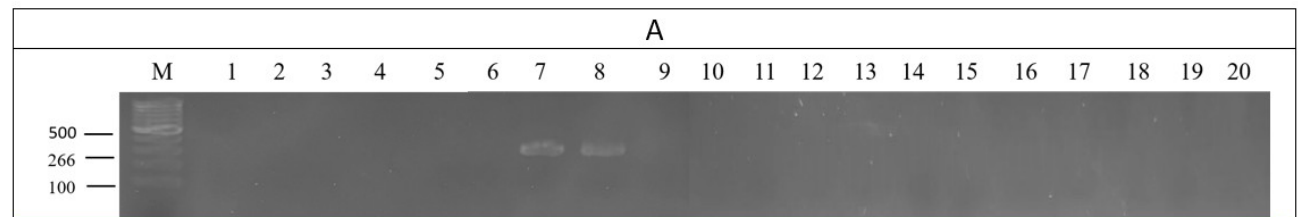


Figure 8. Visual results of DNA sample electrophoresis using primers SIX1a_266_F and SIX1a_266_R with a target amplification product of 266 bp (A) Field samples. (1,2,3: Raja samples), (4, 5, 6: Kepok samples), (7, 8, 9: Cavendish samples), (10-20: Barangan samples)

limited drainage, compaction of the topsoil layer, and high clay content, which promotes water saturation in the soil profile. This is certainly favorable for pathogens and inhibits the growth of the banana root system, thereby increasing susceptibility to pathogen infection. In contrast, areas classified as moderately susceptible have well-drained soils, with a predominantly sandy texture and better soil drainage conditions. Field surveys (Table 3) showed significant variations in incidence among cultivars ($p < 0.001$), with the Barangan cultivar in Belapunranga showing the highest incidence (18.33%), and the Raja and Kepok cultivars in Borisallo showing complete resistance (0%). These differences confirm the variation in cultivar susceptibility to Foc, as reported by Dita *et al.* (2018) and Maryani *et al.* (2023). The low incidence in Cavendish in Bontokassi (0.17%) indicates the possibility of good management practices or soil conditions that are less conducive to pathogens. The susceptibility of the Barangan cultivar is consistent with the report by Ismaila *et al.* (2023), who found that AAA and some AAB varieties are susceptible to race 4. Conversely, the low incidence of Foc TR4 in Cavendish at Bontokasi (0.17%), despite this variety being susceptible to Foc TR4, suggests that infection can be prevented with good agronomic practices and optimal field sanitation.

The identification of race 4 through the volatile odor test (VOT) (Table 4) has been proven effective

as a phenotypic screening method, characterized by the production of aldehyde odor (Odoratum) in 10 isolates from the Cavendish, Kepok, and Raja cultivars after 3 days of incubation. These results confirmed the specific biochemical characteristics of Foc race 4. This is in line with the study by Nasir *et al.* (2003), who stated that if Foc isolates on rice culture media produce aldehyde aroma (Odoratum), then the isolate is Foc race 4. The production of aldehydes in VOT is the result of secondary metabolic activity specific to Foc Race 4, in which the alcohol dehydrogenase enzyme oxidizes alcohols into aldehydes during fermentation in the rice medium (Mooren *et al.* 1995). This is consistent with the biochemical characteristics of Foc race 4 and validates VOT as a reliable initial screening method in the field. However, the accuracy of this method is limited to the race level and is unable to distinguish between virulent subtypes within race 4, particularly between subtropical race 4 (STR4/VCG 0120) and tropical race 4 (TR4/VCG 01213/16). These two subtypes have different virulence, with TR4 being virulent under all environmental conditions, whereas STR4 virulence is limited to subtropical regions, thus requiring molecular confirmation using TR4-specific markers (Ploetz 2015).

PCR detection using the four sets of primers, FocEf3_F/FocEf3_R, SIX10a_309_F/SIX10a_309_R, SIX13c_343_F/SIX13c_343_F, and SIX1a_266_F/SIX1a_266_R, showed that only two primers succeeded

in amplifying the target DNA: FocEf3_F/R (General Foc) and SIX1a_266_F/R (TR4 specific), and these primers were only found in samples of mature banana plants derived from the tubers at the Bontokassi location. FocEf3 and SIX1a primers detected two Cavendish samples from Bontokassi, indicating that both samples were infected with Foc TR4. Dual detection with these two different markers strengthens the validity of the results and improves diagnostic accuracy. The sensitivity of detection with the SIX1a_266 primer was 5.3%. This finding is crucial, given that TR4 poses a serious threat to global banana production (Drenth & Kema 2021). The SIX1 (secreted in xylem 1) gene is an effector protein that plays a role in TR4 pathogenicity by weakening host plant defenses.

Meanwhile, SIX10a_309_F/SIX10a_309_R and SIX13c_343_F/SIX13c_343_R primers did not show positive detection results in any of the growth phases. This difference is likely due to the lack of expression of the target gene in the tested samples. These results indicate that although SIX genes are often used as molecular markers for Foc, not all SIX genes are equally informative for all isolates or samples, consistent with the findings of Carvalhais *et al.* (2019), who recommended the use of primer combinations to improve detection accuracy.

The differences in detection results between the primers cannot be interpreted as differences in sensitivity because each primer targets a different gene. The SIX1a primer targets the SIX1 effector gene specific to TR4; therefore, a positive detection with this primer confirms that the isolate belongs to the VCG-01213/16 (TR4) group (Carvalhais *et al.* 2019). The FocEf3 primer targets the TEF-1 α gene, which is universal for Foc, but detection with this primer requires a higher concentration of target DNA or better DNA quality than the other primers tested. The consistency of the results between the VOT and PCR methods reinforces the validity of identification however, the differences in detection results between primers indicate the need for a layered diagnostic approach for comprehensive surveillance. Further research is needed to understand pathogen population dynamics and develop location-specific integrated control strategies to mitigate the economic impact of fusarium wilt in Indonesia.

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