Panama Disease (TR4), an unstoppable threat

What is the impact of TR4 in the banana market?

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Fusarium wilt disease, also known as Panama disease, has been a major problem of global or banana production for more than a century. This disease is caused by a soil-borne fungus called *Fusarium oxysporum f. sp. cubense* (Foc) Tropical race 4 (TR4). Panama disease is a world wide pandemic that has led to economic losses worth millions of dollars in the banana market. Given the catastrophic impact of these disease, an early detection is crucial. This article summarizes the state of the art of Panama disease and also methods to detect it in plant and soil.

An introduction to Panama disease

Panama disease is one of the most damaging diseases for banana cultivars. Panama disease is caused by a fungus, called *Fusarium oxysporum sub-race cubense* Tropical Race 4 (Foc TR4) (Ploetz, 2015).

Foc TR4 is a soil-borne pathogen able to remain inactive in the soil and survive the absence of a suitable host for longs periods of time in the form of survival spores. Reservoirs of Foc can be found in the form of chlamydospores or in infected non-economical hosts such as weeds (Dita et al., 2018).

Once Foc TR4 gets in contact with the target host, it develops in the cortex and epidermis of the root. At the same time, the mycelium progressively infects the vascular tissue of the plant, specifically the xylem, those interfering with nutrient uptake and water transport. Eventually, the fungus destroys the vascular system, thus leading to the death of the banana plant (Chunqiang Li et al., 2017; Chunyu Li et al., 2011). Other plants get infected either via connection through the vascular tissue or due to proximity with the infected plant, thus starting the cycle again (Figure 1, Dita et al., 2018).

Figure 1. Schematic representation of the infection cycle of Foc TR4 (Dita et al., 2018)

Foc TR4 over the years

Foc TR4 was first reported in Taiwan in the 70's and it quickly spread to other south Asian counties like Philippines, due to human labor and shipping of contaminated bananas. In the following years Foc TR4 was also detected in Australia and Africa (Dita et al., 2018).

Extreme efforts have been taken to stop the spread of this catastrophic disease. However, in the past two years Foc TR4 was first detected in South America.

Colombia detected their first case of Foc TR4 in 2019 (García-Bastidas et al., 2020). In 2021, Peru also found traces of Foc TR4 within their frontiers and Venezuela announced a suspect of Foc Tr4 (freshfruitportal, 2021b, 2021a)

South America and the Caribbean are the biggest exporters in the world market with an estimated 80% of the total shipment of bananas, in a business that generates about US $6 billion (FAO, 2019). Hence, the presence of Foc TR4 could have devastating consequences for the South American economy.

Consequences of Foc TR4 in your farm

There is no current fungicide to eliminate Foc TR4 in the field. Current methods to treat infected soil relay in soil and plant burning. However, Salacinas (2019) suggested that this technique may not be sufficient to remove all Foc TR4 traces from soil. In addition, Foc TR4 can be easily disseminated in the field due to the workers transit and the use of uncleaned working equipment (Figure 2, FAO, 2021)

Figure 2. Farm infected with Panama disease.

In most cases, the presence of Foc TR4 not only leads to a significant yield loss, but also to the temporal closure of the farm. Annual economic losses for Indonesia are estimated in US $121 million, US $253 million in Taiwan and US $14 million in Malaysia (FAO, 2019).

Ways to detect Panama Disease

In the field, one of the first symptoms of Panama Disease include a leaf browning and reduction in productivity. However, these symptoms are like other abiotic stresses like low water uptake. To confirm the presence of Foc TR4 farmers would need to sacrifice the banana plant and observe the necrotic vascular system (Figure 3, Promusa, 2019).

Figure 3. Necrotic vascular system of banana plant infected with Foc TR4 (Promusa, 2019)

Morphological identification of Foc TR4 has many disadvantages, but one of the most important ones is that it does not allow an early disease detection. Given the fast spread and infectivity of the disease, an early detection is critical.

Traditional quantification of Foc TR4 spores in infected material including plant and soil, is usually done by plate dilution, also called plating (Dita et al., 2010). However, this technique is a highly time-consuming procedure that can take up to 7 days to give results (Catambacan & Cumagun, 2021).

The most popular methods to detect Foc TR4 are techniques based on the amplification and quantification of DNA, such as the widely used Real-Time PCR assay (M. A. Dita et al., 2010; Lin et al., 2013, 2016; Yang et al., 2015). In particular, Real-Time PCR presents several advantages compared to other methods based on PCR. Real-Time PCR is highly sensitive, and it allows an easier automation while allowing the amplification of multiple samples (Bleve et al., 2003).

In ClearDetections, we commercialize a Real-Time PCR kit for the detection of Foc TR4 with an improved version of the primers reported by Dita et al., 2010.

Figure 4. ClearDetections Real-Time PCR Diagnostic Kit, Panama Disease (Foc TR4).

The primers of our PCR for the detection of Foc TR4 are based on specific single nucleotide polymorphisms (SNP) present in the Intergenic Spacer (IGS) region of Foc TR4. So far, Foc TR4 Real-Time PCR kit can work complementary with DNA extracted from infected plant material, showing high sensitivity and specificity. This method is reported by the "Guía Andina Para El Diagnóstico De Fusarium Raza 4 Tropical (R4T)" for the detection of Foc TR4 (García-Bastidas et al., 2020).

Alternatively, research has focus in the molecular detection of Panama Disease. Wageningen University & Research reported the development of a rapid in-field test for Foc TR4, based on the Loop-Mediated Isothermal Amplification assay (LAMP) (Ordóñez et al., 2019).

Development on Panama Disease detection in soil

In the field, Foc TR4 can survive in the form of spores called chlamydospores. These spores are often found in the soil area surrounding the infected plant. Unlike asexual spores called conidia spores, chlamydospores can survive in the soil despite the lack of a suitable host, or extreme drought. Infected soil can attach to shoes and tools, thus dispersing Foc TR4 around the field. Hence, the accurate detection of spores in soil is crucial to avoid the spread of the disease (Bai et al., 2019).

Current methods to detect Foc TR4 in soil relay on plating, which is not only time consuming but also limits the amount of samples that can be tested due to space constrains. In addition, spore quantification by plate dilution may also result in an underestimation of the Foc TR4 concentration, as not all the spores are recovered from the sample (Yadav & Singh, 2017).

Molecular diagnostics of soil samples could provide a more sensible, accurate and fast detection of Foc TR4. Direct DNA extraction and amplification via Real-Time PCR from soil will allow the detection of Foc TR4 in bigger sample volumes in a matter of hours (Yadav & Singh, 2017).

However, DNA extraction and purification from soil can be challenging. DNA extracted from soil generally has low concentration, presence of impurities, and inhibitors such as humic and fulvic acids, which heavily affect the performance of the PCR. Also, soil composition greatly varies between samples, thus affecting the final yield of the DNA extraction (Wilson, 1997; Zhou et al., 1996).

In ClearDetections, we are investigating on improving current methods on the extraction and purification of Foc TR4 DNA from soil samples. In addition, we aim that our research can infer the effect of different soil compositions on the purification and detection of Foc TR4.

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