

Genetic diversity of *Ralstonia solanacearum*, causal agent of banana bacterial wilt, and its control

Introduction

Plantain is a staple food in the Colombian household basket. Exports are an important source of foreign exchange for the national economy. The crop is grown on 350,000 ha throughout Colombia. Production exceeds 2.5 million tons per year, with 99% destined for the domestic market and the rest for export. There does however appear to be considerable potential for export as world demand is increasing. For the major production areas—the Coffee Zone, inter-Andean valleys, Colombian Caribbean Region, and the Eastern Plains—the crop proved to be a viable alternative at times when other crops have faced serious problems of sustainability. Of particular importance in areas with high levels of unemployment is its high labour requirement of 100 working days per year per hectare. ,

The central Coffee Zone of Colombia (Departments of Valle, Risaralda, Quindío, and Caldas) is the biggest plantain producer in the country, with an estimated area of 40,000 ha. In this region, together with other plantain-producing areas, associations of small farmers are interested in linking with the market economy and producing cash crops, including plantains.

Moko or banana bacterial wilt, caused by *Ralstonia solanacearum*, is a devastating disease of plantain. It is found throughout Colombia and causes an estimated annual loss of about US\$5.8 million. The problem is compounded by environmental deterioration caused by indiscriminate use of toxic substances such as fungicides as control agents.

The only controls that farmers currently use are clean seed and cultural practices that are too short term to be really effective in eradicating or detaining the disease.

Objective

This study aims to protect plantain and banana crops against *moko* on a national scale. If the disease's progress is not stopped, production will deteriorate and may reach the point where it is not economically viable.

Materials and methods

Surveying and sampling. In two samplings, we visited 36 plantain farms in the Municipalities of Montenegro, Quimbaya, La Tebaida, and Armenia (all in Quindío). Each farmer was asked a set of 40 questions. Their answers were used to describe management practices used in plantain and their effect on *moko* disease. To isolate *Ralstonia solanacearum*, the causal agent of *moko*, we took 144 samples, involving plant tissues (137), soil (146), water from reservoirs and streams (18), and insects (3).

Tissue samples. Fragments were cut from tissue samples in which symptoms of the disease were evident and from healthy tissue. The fragments were placed in beakers sealed with gauze and put into de-ionized water for 15 min. They were then disinfected by immersing in a solution of 1% NaOCl for 2 min, and washed in sterilized distilled water to remove the solution. The tissue fragments were then macerated in a sterilized solution of 1 L of distilled water; 50 mM phosphate (pH = 7.0); 4.26 g Na₂HPO₄; and 2.72 g KH₂PO₄. The macerated tissue was left for 15 min, before culturing each sample in duplicate in tetrazolium chloride (TZC) medium a differential for *R. solanacearum* and made up of 10 g peptone; 1 g hydrolyzed casein; 0.5 g glucose; and 17 g agar.

A stock solution of 2,3,5 triphenyltetrazolium chloride at 0.5 g/100 mL was prepared and sterilized separately from the TZC medium. At the time of use, 3 mL were added per 100 mL of medium.

Water samples. The water samples were diluted in sterilized distilled water by taking 1 mL of sample water and adding it to 9 mL of distilled water. Dilutions at 10⁻¹ and 10⁻² were cultured in TZC medium.

Insect samples. The insect samples were processed as for the tissue samples described above.

Soil samples. The soil samples were left to dry first by taking them out of the bags in which they were collected and exposing them to room temperature. Once dried, the samples were passed through a 2-mm sieve, and 10 g were taken from each sample and added to an Erlenmeyer flask containing 100 mL of sterilized distilled water. The samples were agitated for 20 min, and dilutions then carried out by adding 1 mL of sample to 9 mL of sterilized distilled water to a dilution of 10⁻³.

From each dilution, 0.1 mL was taken, and dilutions of 10⁻² and 10⁻³ cultured in TZC medium.

Purification. To isolate *Ralstonia solanacearum* we first identified and purified suspect colonies that grew in the TZC medium by culturing them in fresh lots of the medium, using the drop method of culturing. The bacteria were evaluate, using biochemical tests, to see if they belonged to this genus.

Biochemical tests. Once purified in the TZC medium, the bacteria were cultured on nutritive agar to identify *R. solanacearum* bacterias and duplicate samples subjected to the following biochemical tests:

- Oxidase test, to which *R. solanacearum* is positive.
- 3% KOH test to which *R. solanacearum* is positive, indicating that is

gram negative.

Conservation. The strains obtained were conserved, either by lyophilization or kept in inclined tubes containing nutritive agar.

Results and conclusions

Isolates of *Ralstonia solanacearum*. We identified 73 strains of *R. solanacearum* (Tables 1 and 2) from 144 samples, of which 61 (42.3%) were positive. We obtained a larger percentage of isolates from the soil samples, indicating that the bacterium is probably found in abundance in soil.

In contrast, we obtained a smaller percentage of isolates from the tissue samples, probably because either the plants were in an advanced state of infection or saprophytic bacteria that grow in sick tissues had inhibited the pathogen, hampering its isolation.

Soil samples that were positive for *R. solanacearum* were from around plants infected with *moko*, at 0-30 and 60 cm deep, 5 and 10 m up the slope, and 10 and 20 m down the slope from the sampling focus. The bacterium was also isolated from soil treated with formol and covered with plastic and from soil treated with formol 1 year ago.

The pathogen was also detected at sites that (1) had not been under plantain cultivation for 6 months, and 2 and 4 years, and (2) had been treated with Basamid®.

Table 1. Obtaining isolates of *Ralstonia solanacearum*, causal agent of banana bacterial wilt, from different tissues of infected plantain plants.

Tissue	Samples with <i>R. solanacearum</i> (no.)	Samples with no <i>R. solanacearum</i> (no.)
Stems	5	8
Seedling	1	1
Rachis	1	0

Table 2. Obtaining isolates of *Ralstonia solanacearum*, casual agent of banana bacterial wilt, from water samples.

Sample	Samples with <i>R. solanacearum</i> (no.)	Samples with no <i>R. solanacearum</i> (no.)
Stream or spring	3	2
Lake	1	0

Puddles on light-plane runway	0	1
Water supply system	3	5

Ralstonia solanacearum was also isolated from the following field plants and crops: cadillo, *Emilia* spp., Ciperaceae, lechuguilla, nightshade, arracacha, and maize.

Surveys. The farmer surveys (Table 3) indicated that:

- Over the last 5 years, they have had plants infected by *moko*.
- Tools, transport of seedlings, and stream water disseminate the disease.
- Very few control methods reduce pathogen inoculum in infected soil.

Table 3. Incidence of *moko* or banana bacterial wilt (*Ralstonia solanacearum*) and management practices in the Department of Quindío, Colombia.

Diagnosis	Value
Study on losses	
Interviewed farmers (no.)	21
Banana farmers reporting <i>moko</i> (%)	95
Area infected by <i>moko</i> (%)	11
Increases in infected area over the last 5 years (%)	43
Isolates of <i>R. solanacearum</i> obtained (no.)	73
Recommended management practices for reducing <i>moko</i> in banana (percentage of use by farmers)	
Treating soil with disinfectant	52
Treating tools with disinfectant	90
Exclusive use of tools for infected plants	57
Few changes in harvest personnel	29
Farmers who apply controls recommended by ICA	86