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Assessing banana stalk susceptibility to pathogens and their virulence ☆

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ARTICLE INFO

Method name:
Assessing banana stalk susceptibility to pathogens and their virulence

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ABSTRACT

The purpose of this protocol is to assess (a) the virulence of fungi on banana stalks and (b) the susceptibility of a banana stalk cutting modality/cultivar to a pathogen. The principle, plant material used, duration and expected results are presented. The materials and the five procedural steps—stalk sampling, inoculum and plant material preparation, pathogen inoculation, incubation, and evaluation of stalk necrosis—are detailed. Inoculum virulence and banana stalk susceptibility to pathogenic fungi are determined by measuring the proportion of necrosis.

Specifications Table

Subject area:	Agricultural and Biological Sciences
More specific subject area:	Plant pathology
Name of your protocol:	Assessing banana stalk susceptibility to pathogens and their virulence
Reagents/tools:	• agar plates with Mathur's medium (for 500 ml: 1.25 g $MgSO_4$, 1.35 g KH_2PO_4, 0.5 g peptone, 0.5 g yeast extract, 5 g saccharose, 7.5 g agar, 500 ml distilled water) • a Malassez counting cell • sterile distilled water • a microscope • 70 % alcohol • a controlled environment cabinet regulated at 20 °C.
Experimental design	Inoculation of pathogens on banana stalks and measurement of the developed necrotic area.
Trial registration	None
Ethics	No statement needed
Value of the Protocol	• First protocol to evaluate the virulence of fungi on banana stalks. • First protocol to assess the susceptibility of a banana stalk cutting modality/cultivar to pathogenic fungi.

Background

Banana is considered a staple food for global food security and a cash crop for income generation in its cultivation regions [1]. However, increasingly virulent and fungicide-resistant fungal pathogens pose a significant threat to banana production in major

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cultivation areas [2,3]. Various banana tissues have been studied to assess the impact of fungal pathogens, including leaves [4], the pseudostem [5], and fruits [6]. However, no studies have specifically investigated the necroses caused by fungal pathogens in the banana stalk.

To address this knowledge gap, the present study aims to investigate fungal pathogen-induced necroses in the banana stalk, using the methodology established by [7] on banana fruits. This research seeks to provide new insights into the pathogenesis of fungal infections in banana plants and contribute to the development of targeted management strategies to mitigate yield losses and improve banana production sustainability.

Description of protocol

Principle

A banana stalk is infected by artificially inoculating a pathogen causing stalk rot. The virulence of the fungus and susceptibility of the stalk are assessed by measuring the necrotic area.

Starting material

The method requires mature, freshly harvested healthy banana stalks and fungal cultures of the studied pathogen.

Time estimation

The time required is 30 mins for stalk sampling, two hours for stalk inoculation, 20 mins to assess the proportion of necrosis.

Expected results

The method measures the proportion of stalk area affected by the pathogen(s) and thereby assesses fungus virulence and stalk susceptibility.

Stalk sampling and inoculum preparation

- Step 1. Stalk sampling
  - For each cutting modality/cultivar, take banana stalk samples from 20 harvested bunches.
- Step 2. Inoculum preparation
  - Transfer a small plug from a fungal colony, isolated from a rotten stalk, to Mathur's medium plates.
  - *Note*: This applies to any pathogenic fungus affecting the vascular tissues of banana plants, such as *Colletotrichum musae* or *Fusarium*. Fungal cultures should be monosporic and not sub-cultured more than five times (initiate new cultures from frozen conidial suspensions at −80 °C in 30 % glycerine). Store fungal cultures at 25 °C for 10 days.
  - Flood the fungal cultures with distilled sterile water.
  - Filtrate the conidial suspension through a 35 µm sieve.
  - Calibrate the conidial suspension to 10^6 conidia*mL^{-1} with the Malassez counting cell.

Fungal virulence and stalk susceptibility

- Step 3. Preparation of plant material

For each stalk, cut off a 10 cm section between the third and fourth hand using a disinfected knife. Dip each end of the section in 70 % alcohol for 10 ss and place the section one hour in a ventilated room at 25 °C for the alcohol to dry.

- Step 4. Inoculation and incubation

Place a 50 µl droplet of a suspension calibrated at 10^6 conidia*mL^{-1} in the centre of one of the sides. Wait 1 h for the droplet to dry. Transfer the stalks, placed in a perforated polybag, to a controlled environment cabinet regulated at 20 °C, 100 % RH for 7 days.

- Step 5. Scoring system and result interpretation

After 7 days, cut the stalk lengthwise into two equal parts and measure the inner stalk area (a rectangle) (SA) and the inner necrotic area (NA). Calculate the proportion of necrosis: (PN) = (NA/SA*100) (Fig. 1) and compare the different pathogens/cultivars by an analysis of variance.

Fig. 1. Proportion of necrosis (%) = (necrotic area/stalk area) *100.

Limitations

None.

CRediT author statement

TD and OH designed the study. TD conducted data collection, analysis, and manuscript writing. OH, LL, BB, and PB supervised the project, provided methodological guidance, and reviewed the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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